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ACUPUNCTURE IN THE MANAGEMENT
OF INJURY AND OPERATIVE PAIN
UNDER FIELD CONDITIONS

ANNUAL SUMMARY REPORT

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This document reports initial development of two experimental programs to permit quantitative assessment of the feasibility of Acupuncture Analgesia for integration into the present pain control armamentarium under clinical and especially field conditions. One experimental program involves acute Neurophysiological experiments in the cat to permit quantitative characterization of optimal Acupuncture waveform (electrical) or manipulation (manual) and optimal Acupuncture points. These experiments involve the

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study of the influence of Acupuncture stimulation on single neuron responses
in three thalamic nuclear groups (associated with pain pathways) elicited by
tooth pulp stimulation. At present, techniques and facility development are
complete, pulp-elicited thalamic unitary responses are continuing to be cata-
logued, and a definitive Acupuncture protocol has been initiated in the Posterior
Nuclear group, the first of the three thalamic nuclear groups of interest in
which this protocol is being executed. The results show that stimulation of the
Hoku Acupuncture point significantly attenuates thalamic unit activity elicited
by pulp stimulation. The second experimental program involves chronic Behavioral
experiments in the cat to permit a direct verification of the feasibility of
Acupuncture analgesia in an animal preparation to avoid interpretive complications
of placebo reactions, hypnotism, etc. This model also permits immediate testing
of the perceptual efficacy of conclusions from the acute experiments, and by the
placement of temporary blocks or permanent lesions, the study of pathways and mech-
anisms to aid in optimization of effectiveness. At present, all procedures of
animal selection, chronic surgical implantation, and animal training to the final
stage of the Threshold Titration program are operational, as is an Automatic Shaping
electronics system for control of all experimental stages in the program. Defini-
tive Threshold Titration protocols involving Acupuncture stimulation are to be
initiated in the immediate future.

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ABSTRACT

Acupuncture works! It has been unequivocally demonstrated that there are significant Acupuncture effects over and above such complicating phenomena as placebo reactions, distraction, and hypnosis. Our laboratory is specifically assessing the feasibility of Acupuncture for the control of injury or operative pain under field conditions, where its attributes become particularly attractive.

The present document reports the results of our effort for the last eight months, involving both Acute and Chronic (behavioral) Neurophysiological experiments in animals. Initially, field potential recording techniques were used to map the general area of the thalamus of cats in which responses elicited by tooth pulp stimulation could be identified. Based upon these results, three general areas of pulp-elicited responses were delineated. Subsequently, we developed single-unit recording techniques and obtained preliminary characterizations of extra-cellular single-unit responses elicited by pulp stimulation in all three of the abovementioned nuclear groups. Based upon the latter information, a series of experiments were initiated which included Acupuncture stimulation. As requested, these experiments were designed for a quick demonstration of overall model feasibility. The Hoku Acupuncture point was used, because this is the most popular point employed for orofacial analgesia. We have also selected one particular area of the thalamus for initial scrutiny, to provide a quantitative demonstration of feasibility and the effective evaluation of Acupuncture effects at the earliest possible date. The area selected for particular scrutiny was the Posterior Nuclear group of the thalamus. It was found that Acupuncture stimulation significantly altered stimulus-related activity, while spontaneous activity was not significantly altered. Furthermore, the Acupuncture effects greatly outlasted the period of stimulation, but, in many cases, activity tended or actually returned to normal by the time recordings were terminated. The data to date provides a powerful demonstration of the feasibility and utility of the experimental model, because the thalamic data can be directly used to derive a quantitative index to evaluate Acupuncture effects, a model which can then be used to sort out optimal stimulus parameters.

In the Chronic (behavioral) experiments, we have designed a program in which direct quantitative measurements of pulpal pain thresholds can be assessed at the perceptual (not reflex!) level in behaving cats, using pulp stimulation alone (control) and during the concurrent administration of Acupuncture stimulation. Following initial animal selection and surgery, the training procedure involves establishing a bar-press response to escape tooth pulp stimulation. This response is then generalized through a minor behavioral modification to a point where the animal will report pulpal thresholds to noxious stimulation in a "Threshold Titration" experimental paradigm. To date, we have designed an Automatic Shaping procedure, and demonstrated efficient behavioral modification from footshock (used for initial animal selection) to pulp stimulation, and the requisite behavioral modification from pulp stimulation to the Threshold Titration paradigm. We have also completely redesigned the experimental facility to permit either footshock or pulp stimulation to be used as the aversive stimulus, to avoid various contingencies impairing experimental success, and to provide for minimal restraint of animal mobility. Furthermore, all surgical procedures for the implantation of pulp and Acupuncture electrodes and installation of the head pedestal for electronic communications are completely operational. We are presently in the process of conducting surgery on several animals which will then be immediately introduced to the "Threshold Titration" paradigm.

Acupuncture Analgesia exhibits definite potential for fulfilling certain roles within the general medical armamentarium for pain control. Acupuncture Analgesia also possesses advantages bearing particular significance in terms of increased manpower efficiency, reduction of hazards, adaptability to field use, and enhancement of effectiveness of various therapeutic procedures. Furthermore, the feasibility of these objectives are being quantitatively assessed at greatly reduced cost by cross-utilization of skills and facilities in a multidiciplinary team effort directed to various pain mechanisms and treatment stategies.

CONCEPTUAL SUMMARY

Pharmacological agents and techniques presently available for local or regional pain control possess certain drawbacks and contraindications. Nerve block or local infiltration is usually effective, but disadvantages include long induction and recovery times, and such contingencies as tissue damage, infection, and toxic or allergic reactions. Inhalation or intravenous agents for general anesthesia provide more extensive control of pain and anxiety, but potentiate the disadvantages of more localized procedures and add prolonged perturbations of various physiological parameters. General Electroanesthesia has undergone extensive international study, and, although plagued with certain drawbacks, may finally be exhibiting potentialities for manageable utility. Regional Electroanalgesia, a new procedure under investigation which shows much promise, has not yet reached operational status. The Electroanalgesic techniques, both local and general, are attractive, because they circumvent most problems which have continuously hampered the use of injected and inhaled pharmacological agents.

In common with the various forms of Electroanesthesia or Electroanalgesia, Acupuncture circumvents most problems encountered with pharmacological agents. As previously mentioned, Acupuncture materials are extremely portable, and allergic or toxic reactions are unknown. The technique has potential use, with limitations, by nonprofessional aide-level personnel under emergency conditions. Using mechanical induction (twirling the Acupuncture needle), infection and tissue damage remain as realistic contingencies, but the much more attractive non-invasive electrical stimulation of Acupuncture points is likely to be feasible, thereby totally circumventing all necessity for tissue penetration. Also, Acupuncture blocks pain, not consciousness, allowing the patient to cooperate during transport or surgical procedures. In addition, recovery is reported to be uneventful, there is no equivalent of a drug hangover, all senses except pain are normal, and vital physiological parameters are not only uncompromised but supposedly are actually actively maintained both during and following surgical procedures. The latter fact makes Acupuncture quite attractive for use on certain high-risk patients.

Acupuncture has certain disadvantages, but they are overwhelmed by the above-mentioned advantages. In many cases, extended induction periods are required. Also, Acupuncture is ineffective in some people and only partially effective in others. Finally, some studies have revealed a possible relationship between the "suggestability" of the subject and subsequent Acupuncture effectiveness. The latter point, if true, would not be surprising, because the effectiveness of any analgesia agent contains placebo components, at least at certain concentrations. Nevertheless, it is important to note that there is absolutely no evidence for any neurological differences which might subserve the above-mentioned contingencies, and they may be the result of inadquacies of technique. In this regard, present techniques are not only diverse but quite inconsistent. Therefore, overall, Acupuncture shows marked potentialities as, minimally, an adjunct to present therapeutic procedures.

Not only are the physiological mechanisms of Acupuncture unknown, but even the traditional numbers and locations of "points" are undergoing contemporary evolution. Therefore, traditional descriptions are highly suspect, and, because of this fact and to insure optimal potentialities for success, we feel a balanced approach along three distinct but highly interrelated avenues is mandatory. We have initiated both an Acute Neurophysiological and a Chronic (behavioral)

Neurophysiological experimental series. These two experimental series in animals are essential to permit rapid sorting of experimental variables and to generate data necessary to obtain official sanction for human studies. The two animal experimental series will eventually be followed by a third series involving human subjects, to verify conclusions and techniques developed in the animal investigations. Tooth pulp will be used as a source of experimental pain, because it is easily accessible, it is classically considered to be a source of pure pain, and is readily adaptable to all three experimental series. The primary Acupuncture points which presently enjoy maximal favor in terms of the control of orofacial pain (there are three; see below) will be thoroughly tested singly and in various combinations to insure effective and thorough evaluation.

Acute Neurophysiological Experiments are required to identify effective animal Acupuncture points, and, subsequently, to optimize the effect in terms of "points" and waveforms, and, for purposes of safety, to establish the maintenance of reasonable physiological homeostasis (in conjunction with the Chronic Neurophysiological experiments in animals). The Acute experiments will also permit the quantitative comparison of manual versus electrical Acupuncture. Although the above information could be gained from Chronic models, it is essential to include Acute experiments, because many of the experimental variables can be sorted much faster using an Acute experimental model. For example, the long induction times involved in Chronic experiments make it difficult to investigate a large number of variables in each experiment. Furthermore, Acute animal experiments provide more flexibility in terms of permissible waveforms and in accurately and quantitatively identifying effective activation of Acupuncture points. Chronic experiments lack an effective criterion for Acupuncture "point" activation, and, therefore, do not provide a means to evaluate whether unsuccessful findings are the result of ineffective "point" activation or if the Acupuncture is actually ineffective. The Acute Neurophysiological data is also much more closely related to the general Neurophysiological knowledge of pain mechanisms, and therefore permits better correlation with previous physiological information. The basic approach employs single-unit recording techniques in specific brain centers suspected or identified as providing an index of the effects of Acupuncture stimulation on tooth pulp pathways involved in perception. Certain of these protocols will be accompanied by continuous monitoring of various indices of cardiovascular and metabolic parameters, to provide preliminary documentation of certain aspects of safety.

Chronic (behavioral) Neurophysiological experiments in animals provide the main framework for the entire program. The Chronic experiments are the key link between the Acute Neurophysiological studies and ultimate human work, because the Chronic Neurophysiological experiments permit tracing of pathways and mechanisms (using chronic recording or lesions) in the same animal in which behavioral effects of Acupuncture stimulation on pain perception can be quantitated. Also, behavioral situations permit the effectiveness of Acupuncture to be examined free from placebo effects, distraction, hypnotism, and various other contributing factors which are plaguing present human investigations of Acupuncture. The Chronic studies are required initially in conjunction with the Acute experiments to ide if Acupuncture points and to demonstrate feasibility. Subsequently, these experiments are indispensible to test the use of the specific Acupuncture points in various combinations and the effects of various stimulation waveforms on actual perception, to test psychological effects, and, in conjunction with the Acute experiments, to test optimal administration parameters and verify safety and physiological homeostasis. Fundamentally, the Chronic preparation will

simultaneously involve the use of chronic implants for electrical stimulation of the test tooth pulp and of specific Acupuncture points, with the animal being tested in a behavioral paradigm for perceptual sensitivity to pain (as opposed to reflex flinching or other motor responses). Certain experiments will be accompanied by chronic recording from various brain centers, by repeated experiments following the placement of specific lesions, and by the recording of various physiological variables concerned with safety documentation.

Eventually, Human experiments will provide the ultimate test of all physiological, psychological, perceptual, and administration protocol characterizations produced in the Acute and Chronic Neurophysiological experiments. The basic procedure will be to test the ability of Acupuncture stimulation to modify the perceptual experience elicited by electrical stimulation of intact teeth, using Signal Detection Theory formats of experimental design to separate true changes in sensory information inflow to the brain from alterations in response criteria of the subject (mental set, anxiety level, hunger, etc.). Such Human experiments are programmed for inclusion after the majority of the Acute and Chronic experiments have been completed, and no further discussion of human experimentation is included in the present document.

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INTRODUCTION

Acupuncture Analgesia is effective. Although contributing phenomena such as placebo reactions, distraction, conditioning, or hypnotism (1-6) may occasionally play a role, careful clinical (1,7,8) and laboratory (1,2) studies in humans and the demonstration that Acupuncture Analgesia works in animals (1,9-11) establishes unequivocally that Acupuncture treatment strategies have true analgesic effects.

In spite of the above results, the concepts which embody our traditional medical knowledge and theories have been deficient in their ability to offer a logical mechanistic hypothesis to explain Acupuncture Analgesia (7,12). Nevertheless, Acupuncture is receiving considerable international attention due to advantages that include portability, infinite shelf-life, and complete freedom from toxic or allergic contingencies associated with present pain-control techniques (10,12). Cardiovascular and respiratory physiology are unaffected, which is particularly desirable in certain high-risk patients. Finally, electrical Acupuncture stimulation is easily administered and requires less attention than presently used anesthetics. This method of Acupuncture may afford noninvasive application (4) and potential opportunities of administration by nonprofessional personnel in emergency situations.

We have initiated a major program to define the feasibility of employing Acupuncture Analgesia for the control of injury or operative pain under field conditions. Initially, the program involves the concurrent evaluation of two experimental models, involving Acute Neurophysiological experiments in animals and Chronic Neurophysiological experiments in animals, respectively. Acute Neurophysiological experiments are required to identify effective animal Acupuncture "points", to permit the rapid sorting of various experimental variables, and to define permissible limits of stimulation for the Chronic experiments and eventual human studies. Many of these factors are the direct result of the increased flexibility afforded by Acute experiments, in terms of permissible stimulation parameters, enhanced control over experimental variables, and the fact that quantitative criteria can be established to verify effective activation of Acupuncture points. The basic approach is to utilize standardized well-established Neurophysiological recording techniques, described in detail below. Chronic (behavioral) Neurophysiological experiments provide the key link between Acute Neurophysiological experiments and Human investigations, because they permit collection of definitive data related to pathways and mechanisms in the same animal in which behavioral effects of Acupuncture stimulation on pain perception can be quantitated. Chronic experiments are also invaluable because they avoid the complications of placebo reactions, hypnotism, distraction, or conditioning, and permit, in combination with the Acute experiments, the definition of permissible limits of safety. The validity and applicability of this model will be established first. Subsequently, it will be used to identify optimal waveforms for three critical Acupuncture "points" associated with orofacial pain control. The same model will then be used to study the use of the three Acupuncture points in various combinations and to document safety. More details of the rationale of the dual approach using Acute and Chronic experiments in animals is given in the Conceptual Summary. The com-

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bination of complementary and confirmatory information obtained from these
two experimental series will provide a powerful information base upon which
the feasibility and potential ultimate utility of Acupuncture as a therapeu-
tic technique can be defined. This combination will also provide data required
to obtain sanction for detailed quantitative evaluation of the therapeutic
utility of Acupuncture Analgesia in humans and to solve clinical engineering
problems.

ACUTE NEUROPHYSIOLOGICAL EXPERIMENTS

The evidence is clear that there is a definite analgesia component to Acupuncture, over and above contributing factors such as placebo reactions, distraction, conditioning, and hypnotism (1,2,7-11). In our overall program directed to the assessment of the therapeutic utility of Acupuncture Analgesia, Acute Neurophysiological experiments are required to permit the flexibility of surgical access and recording techniques not readily available in Chronic experiments and unacceptable for studies in humans. They also permit the rapid survey of multiple experimental variables. The key to the approach in the Acute experiments is that Acupuncture has also been shown to be effective in animals (1,9-11), a fact which further substantiates the existence of a true analgesia component to Acupuncture.

The central features of our Acute experimental model involve the identification of a locus (or loci) in the brain which has the following characteristics: a), activity is indicative of noxious stimulation of the tooth pulp; b), there is a suspected direct participation in the transmission of information to perceptual (not reflex) centers; and, c), activity is attenuated by Acupuncture stimulation. This general experimental design is depicted in Figure 1. Activity from thalamic units meeting all three criteria are designated "index" responses, and such responses will then be employed to quantitatively evaluate the effects of specific Acupuncture protocols.

Tooth pulp stimulation is used as the source of experimental pain, because this structure is readily accessible, is representative of orofacial pain in general (13), and because the perceptual response in humans (14,15) and apparently in animals (16,17) is always painful, regardless of the nature of the stimulus (thermal, mechanical, chemical, or electrical). Furthermore, it has been chosen because of correlation with previous data from the literature (13,18-25) and from work within our laboratory (26-30). Rectangular stimulus pulses of 0.1 ms duration and suprathreshold intensity are employed. Stimuli are applied in the bipolar configuration to ensure limitation of current within the pulp chamber proper (27,31), and constant current stimulus output units are used to ensure uniform stimuli in the event of alterations of pulpal impedance (32). A short train (burst) of stimulus pulses, as opposed to single shock stimulation, is employed, because there is significant evidence from the literature (33,34) and especially from our laboratory (35) that effective activation of units at high levels in the central nervous system elicited by pulp stimulation have much lower thresholds when train stimulation protocols are employed.

The precise point for recording the best index of nociceptive activity and interactions is unknown. However, a reasonable approximation can be made based upon present knowledge. There are at least two major ascending systems for pain, heavily colored by a third pathway which modulates the other two (34). All three pathways exhibit anatomical proximity in the thalamus, but involve discrete anatomical and physiological nuclear groups in that structure. Also, all three thalamic nuclear groups are located at a high level of integration, permitting many interactions to have occurred at lower levels. A few prior studies have implicated Acupuncture effects on nociceptive units in the thalamus (36-38). Therefore, our approach is to examine

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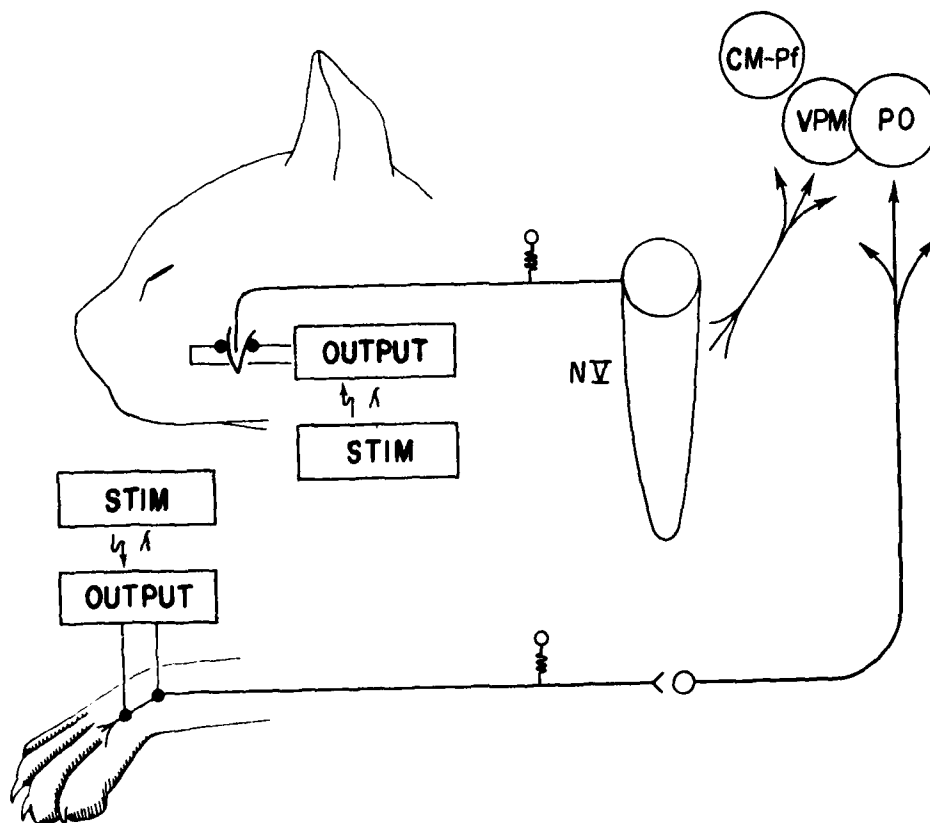
Fig. 1. Diagramatic representation of the experimental model for the Acute
Neurophysiological Experiments. The three thalamic nuclear groups
from which single unit recordings were obtained are indicated in the
upper portion of the figure (PO, VPM, CM-Pf), as is the Trigeminal
Sensory Complex. The figure is reproduced from last years annual
report. VPM ≈ VB; CM-Pf ≈ CM.

in detail single units in all three nuclear groups at the level of the thalamus involved in nociception and choose one nuclear group as the best, if possible, to use as an index of the effectiveness of various strategies of Acupuncture Analgesia administration.

Relevant Acupuncture points or peripheral nerve sites just proximal to the point (the latter has been shown to result in equivalent effects; ref. 12) are being employed for Acupuncture stimulation. Rectangular stimulus trains of various frequencies, intensities, and pulse durations are being used which exhibit definite potential based upon the present literature and information from our laboratory. Also, Acupuncture points have been selected for study which are the most popular for the control of orofacial pain, based on consultation with the president of the Oregon Acupuncture Association (39). Optimal waveforms will be identified for each individual point, and, subsequently, points in various combinations will be intestigated. To date, experiments have involved bilateral stimulation of the Hoku point.

All experiments are being conducted using standardized Neurophysiological stimulation and recording techniques, and employ the anesthetized cat as the experimental animal. Cats have been used as the experimental animal for economy, availability, correlation with previous work in our laboratory (26-30), and based upon the wide use of the cat as a general model of somatic (20,21) and orofacial (18) pain.

Methods

Animal Preparation and Surgery. The acclimated animal was brought to the experimental surgery after the preoperative administration of atropine sulfate. Endotracheal entubation was accomplished using a short-acting barbiturate. Then, Ethrane-Oxygen was administered via a closed inhalation circuit. Upon reaching a level of deep surgical anesthesia, the animal was maintained at that level using Ethrane-N_2O-Oxygen throughout the entire surgical procedure. This anesthetic combination was employed because it mimicks the effects of Chloralose, classically, the anesthetic of choice in studies of activity in pain pathways (19) and in studies of central nervous system effects of Acupuncture (40). Subsequently, an esophogeal temperature probe and stethescope were immediately placed, an optically coupled ECG (to preserve ground isolation) was attached (Terrasyn Model N-IIIB ECG Isolation Amplifier), and a non-invasive blood-pressure monitoring system was applied to one of the anterior limbs (Hoffman-La Roche Arteriosonde, Model 1010). In addition, continuous monitoring of short-term acid-base balance was maintained utilizing a Beckman LB-2 Medical Gas Analyzer monitoring end-tidal CO_2. Respiration was assisted on demand, or controlled, using a Bird Mark IV-VIII Anesthesia Assistor. Intra-esophogeal temperature was monitored (YSI Model 43TA) and maintained at 38 $\pm$ 0.5 C by means of a heating pad with thermostatically controlled circulating water (Gaymar Temp-Pump System).

Initially, the animal was placed in a stereotaxic apparatus, and two stimulation electrodes were placed in the experimental tooth by methods which we have previously described in detail (27). One electrode was placed in each of two cavities drilled through the enamel to a near pulp exposure. The base of each cavity was filled with Eccobond, the electrode wire (stranded) was packed in place, and the whole complex was sealed in position using a

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non-conductive filling material. One of the two stimulus electrodes was located on the lingual aspect and one on the buccal aspect of the maxillary canine test tooth, each electrode being near to but absolutely isolated from the gingival margin. Both stimulation electrodes were composed of platinum, to reduce the effects of electrode polarization (32). Based upon our data (27) and data from the literature (31), this stimulus configuration effectively limits current flow to the tooth pulp.

The Hoku Acupuncture points have been used in the experiments conducted to date. The Hoku point is the most popular site associated with maxillary orofacial pain (39). The Hoku electrode was placed by surgically exposing the requisite peripheral nerve supplying this point (medial branch of the superficial radial nerve) just proximal to the actual Hoku point, and wrapping the bared stimulus lead wire around the nerve. The indifferent electrode was composed of a syringe needle, and was inserted randomly into a proximal site in the anterior limb. Stimulation of the peripheral nerve supplying the Acupuncture point has been shown to be equally as effective as stimulating the Acupuncture point itself (12). We have been using symmetrical bilateral stimulation of the Hoku point, as this configuration is that which is most popular in the literature and which was advised by our Acupuncture consultant (39).

The surgical procedure required to gain access to the recording site involved the initial reflection of cranial muscles to expose the cerebrum overlying the thalamus. Subsequently, a defect was created in the hard tissue using a dental bur. The resultant defect was then filled with saline agar to afford protection from exposure and to limit cerebral movements induced by circulatory or respiratory pressures. It has not been necessary to induce a pneumothorax or to ventilate particular cerebral ventricles to limit cerebral movements, but we have these options available if required.

Pulp Stimulation. The stimulation used to evoke single-unit activity in the particular thalamic nuclear groups to test pulpal excitability (test stimulation) was administered in the bipolar configuration between the lingual and buccal pulp electrodes. The bipolar configuration was used to limit current flow to the test pulp (27,31). Stimuli were delivered to the two platinum test electrodes from a battery-powered constant-current generator (lab-constructed), triggered by and photically-isolated from one channel of a Grass S-88 Physiological Stimulator. Each stimulus episode was composed of three rectangular pulses of 0.1 ms duration applied at 300 Hz. The intensity of the test stimuli was set at 50 percent above threshold for the particular pulp-driven thalamic unit under investigation.

Acupuncture Stimulation. Acupuncture stimulation was applied electrically, and the bilateral stimulus configuration was employed. The stimulation was applied to the respective point on each side of the body and the corresponding indifferent point located proximally on the same limb. Electrode polarities were arranged such that the cathode was placed at the active point (39). Platinum wire was used for all electrode contacts. Stimuli were delivered to the platinum electrodes from a battery-powered constant-current generator (lab-constructed), in a manner analogous to the tooth pulp stimulation, triggered and photically isolated from a second channel of the Grass S-88 Physiological Stimulator. The intensity for stimulation at the Acupuncture points

was determined by finding that intensity which was just (ten percent) below the intensity necessary to induce visible muscular fasiculation. In the particular case of the Hoku points, this intensity has been found to be approximately 1 ma, which correlates with values used clinically (7,39).

Recording System. The Acute Neurophysiological experiments conducted during the present reporting period included initial experiments to characterize general properties of thalamic units in all three nuclear groups of interest, and the initiation of a definitive study involving the Posterior nuclear group. All recording protocols were initiated by a systematic search of the particular nuclear group in question, using stereotaxic techniques (see below). The electrodes employed were Frederick Haer Epoxy-Coated Tungsten Wire Microelectrodes, with a tip diameter under 1 μ and with an exposed tip of under 5 μ. The recording system involved a Frederick Haer Preamplifier Model 40-20-2, from there to a Tektronix 3A9 differential-input preamplifier with band-pass limitation, a Mentor Model F-60 60 Hz notch filter, and on to a Tektronix 564-B oscilloscope with a 3A3 differential-input vertical amplifier. Permanent recordings were obtained using a continuous recording camera (Model PC-2A, Nihon Kohden).

Experimental Protocol. The procedures employed involved extracellular single-unit recordings from specific thalamic nuclear center(s) concerned with pain conduction. Following surgical preparation of the animal, the electronic stimulation and recording equipment was connected, tested, and adjusted, and the equipment array was connected to the stimulation and recording electrodes.

There are three distinct nuclear complexes of the thalamus that are associated with pain (33,34). All Acute Neurophysiological experiments were initiated by a systematic search of the requisite thalamic nuclear group, using stereotaxic techniques based upon our own experience and information from the literature (19,20). The search for single pulp-driven units was conducted by systematically mapping the thalamic nuclear complex in vertical tracts along medial-lateral and proximal-distal grid coordinates. A given penetration involved the extremely slow (e.g. 1-2 μ/s) manual or hydraulic (Frederick Haer Hydraulic Microdrive) advancement of the electrode tip in the vertical tract during stimulation of the test pulp, using the standard test stimulus parameters (see above). Once a pulp-driven unit was located, an attempt was made (carefully) to optimize response amplitude, and then the physiological properties of the unit were characterized. The parameters which were quantitatively assessed included threshold, latency, amplitude, certain indices of fiber refractoriness, and the nature of the response waveform. All properties of the unitary responses were catalogued for correlation with respective properties of other thalamic units as described in the literature (19,20). It has been our practice to identify neurons as belonging to a particular nuclear group based upon stereotaxic coordinates and on physiological properties. We did not employ histological techniques to verify unit location for economic reasons, but, eventually, we plan to introduce the use of such techniques.

Once a pulp-driven unit had been identified and the characterization of its physiological properties completed, definitive experiments were initiated. Control or baseline data in the absence of Acupuncture stimulation was collected, in which both the responsiveness to pulp stimulation and the nature

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of spontaneous activity of the thalamic unit was recorded. Control (baseline) data was collected for prolonged periods of time (up to 1.5 hours), to permit documentation of the consistency and stability of the experimental preparation. Once the control data had been collected, Acupuncture stimulation was initiated (a train of 0.1 ms pulses at a frequency of 50 Hz and an intensity of 150 percent of pulpal threshold). Acupuncture stimulation was continued for approximately 0.5 hours, to allow for prolonged induction periods noted in certain experimental procedures in the literature (7,41). Upon termination of Acupuncture stimulation, the responsiveness of the pulp-driven thalamic unit was monitored for an extended time interval to document the recovery process.

The method of data acquisition for the Acute Neurophysiological experiments relied on the dot-raster technique of data display (42) recently introduced into Neurophysiological research. The central concept of the dot-raster technique lies in the fact that in recording responses from single units, the occurrence of the unitary response is sufficient, and information regarding waveform is not relevant. Using the dot-raster technique, an individual stimulus episode was represented as a single horizontal sweep on the oscilloscope. No signal was present unless a response occurred, but, each time the unit responded, a dot was displayed at that point in time on the oscilloscope sweep to represent the fact that the unit fired. This procedure was accomplished by modulating the intensity of the oscilloscope beam (z-axis modulation). The first sweep of an extended sweep-stimulus presentation series was presented near the top of the oscilloscope screen. Subsequent stimulus-response episodes were presented as additional horizontal sweeps positioned at successively lower levels on the oscilloscope screen. This technique afforded us the ability to present and store for photography many (typically, 64) stimulus-response trials on a single oscilloscope screen. This sequence of data collection and recording afforded the compilation of voluminous data into a form that was readily available and easily interpreted.

Data Analysis and Interpretation. The thalamic data collected from single pulp-driven units in the Acute Neurophysiological experiments was analyzed to classify their properties relative to other thalamic units (19,20) to provide a direct comparison of the nature of nociceptive units from tooth pulp relative to pain units from other sources and to provide a basis for interpretation of the Acupuncture data. Data from the prolonged control (baseline) period was quantitatively evaluated to assess the stability of our animal preparation in order to verify its validity. Data collected during the application of Acupuncture stimulation was used to quantitatively compare unit behavior in relation to the baseline data, with regard to both spontaneous and stimulus-evoked activity. The data from the recovery period, collected following the termination of the Acupuncture stimulation, was analyzed in a similar fashion. The data was interpreted not only in the light of attenuation of specific responsiveness, but also in terms of the known requirements for temporal and spacial summation associated with interpretive processes in pain pathways. The results of the definitive experiment on the PO units which has been initiated, in conjunction with similar definitive experimental series characterizing CM and VB units, will be used to derive the optimal index (thalamic unitary responses) for further characterization of Acupuncture effects.

Results

Physiological Properties of Thalamic Units. As of the present time, we have recorded from 165 diencephalic units. All units have been found to lie within ± 1 mm of one of the three thalamic nuclear groups of interest, based upon stereotaxic coordinates. The purpose of these experiments was to quantitatively survey the physiological properties of units in the three thalamic nuclear groups and to catalogue these general properties. This data formed the basis for the quantitative assessment of the efficacy of Acupuncture stimulation. A portion of this data was collected prior to the present reporting period.

The physiological properties of single-unit responses from all three nuclear groups (Table I) have consisted of a single spike, or, more commonly, a short burst of spikes. The spikes have been small (40-300 μV) and usually consisted of a negative phase followed by a positive phase of lower amplitude. A few positive-negative and positive-negative-positive spikes were also found but no purely positive spikes have been observed, indicating that the responses were recorded extracellularly from cell bodies rather than axons (43). Latencies were mostly in the range of 15-30 μs and generally varied within 10 percent of the mean value for a particular unit. Mean latencies for the response of a particular unit to monopolar pulpal stimulation have been found to be 10-20 percent shorter than for bipolar stimulation, presumably indicating a more proximal activation of pulpal nerve fibers in or near the root canal of the tooth. Latencies, burst sizes, and threshold to bipolar pulpal stimulation for the units found in CM, PO, and VB are given in Table I. Many individual units responded to stimulation of the ipsilateral mandibular canine (spatial convergence) and to tapping of the tooth, mechanical stimulation of the hard palate, or to the stimulation of other orofacial areas (polymodal convergence). In general, train stimuli were much more effective in eliciting thalamic unitary responses.

Dot-Raster Recording Technique. As described in the Methods section, the dot-raster recording technique permits the presentation of a large volume of data in a condensed and easily visualized form. Details of the dot-raster concept and the mechanics of its operation are also discussed in the Methods section of this report. This technique was implemented early in the present reporting period, concurrently with continued preliminary unit characterization experiments, by construction of a Raster stepper. The latter device permitted imposition of sequential steps in vertical position of the oscilloscope beam at set time intervals. As previously described, each horizontal sweep of the oscilloscope represented one stimulus episode, with a dot representing the occurrence of a single-unit response. The unit responses were isolated from other ongoing activity and noise using a window discriminator (Frederick Haer, Model 74-45-1). Once an individual stimulus response sweep had been completed, the raster stepper then shifted the vertical position of the oscilloscope sweep slightly, and the stimulus response sequence was repeated. In this fashion, many (typically 64) stimulus trials c `ld be superimposed on one oscilloscope screen for photography. In the reported experiments, a dot-raster display was generated every five minutes. Within each dot raster display, one stimulus trial was initiated every second; thus a total dot-raster display represented 64 stimulus-response trials. The dot-raster displays were generated only at five minute intervals, as the data

TABLE I

Nucleus	PO(N=30)	CM-Pf(N=18)	VPM(N=31)
Mean Latency (msec)	18.40	28.22	30.14
Mean Number of Spikes in a Burst	2.42	3.11	2.92
Mean Threshold (mA)	.14*	.25*	.17*

Properties of Single Thalamic Neurons in the Three Nuclear Centers Associated with Nociception.

* Mean Threshold values based on sample sizes of 24, 8, and 7, respectively. Earlier data for these values were disregarded due to electronic recording which invalidated the results.

would otherwise have been overwhelming. We are presently attempting to generate a system by which we can digitize the dot-raster data on tape, permitting off-line computer processing. If funds for this system can be identified, future dot-raster data will be collected on a continuous basis.

Definitive Acupuncture Data from PO. Upon completion of the baseline characterization of the physiological properties of thalamic units, preliminary studies were initiated using Acupuncture protocols. Initially, we investigated units in all three thalamic nuclear groups to quickly estimate which might be the best for an initial experimental series. This initial experimental series was required to document the feasibility of the technique. Of necessity, the initial series was limited to one nuclear population to statistically optimize the data between individual experiments. We chose PO for the initial definitive series, because PO units were easily located and held for prolonged periods of time. Also, this nuclear group has been associated with aspects of pain other than pure somatotopic location (20,33,34). PO seems more likely associated with temporal summation and polymodal responsiveness, characteristics which are important in the affective or "hurt" component of pain.

As described in the Methods section, the experimental format was composed of a control period, a period of Acupuncture stimulation, and a recovery phase. Figure 2 illustrates the nature of the raw data. Three dot-raster scope displays are presented, each representing 64 stimulus-response episodes. The upper display is an example selected as representative of the control or baseline period. Activity on the left half of the display represents the spontaneous activity prior to stimulation. The tooth stimulus was applied at the center of the screen (a train of three pulses; see Methods). In many cases, stimulus artifact intensities were within our window discriminator setting, and, as in the case shown here, were registered as dots in a fashion similar to thalamic single-unit responses (the three vertical columns of dots just to the right of the center of the display). The data for this particular unit during the baseline period showed the typical burst-type response pattern of thalamic units, which then tapered off markedly after approximately 20 ms. During Acupuncture stimulation (center display), the results indicated little change in spontaneous activity of the unit, but a significant decline (although not elimination) of the stimulus-elicited burst-type activity. Following termination of Acupuncture stimulation (lower display), the data indicated essentially full recovery of the burst-type activity, and, again, little change in the level of spontaneous activity relative to the baseline period. Recovery was not always complete by the time of termination of the experiment, but the single-unit responses almost always showed a progressive tendency to return to the control level. If the collection of data during the recovery period had been continued for longer intervals, it is likely that full recovery could have been documented.

The previous material described the data of individual dot-raster displays. Figure 3 summarizes all of the data from one complete experiment (the same experiment as the data of Figure 2). The data is presented in Time-Histogram fc ut. The height of each bar represents the number of times the unit fired within the indicated post-stimulus time interval (10 ms bins). The zero level on the graph does not represent the total lack of activity but represents the fact that the average level of spontaneous activity for that period had been subtracted from the data. The left third of the figure represents the baseline

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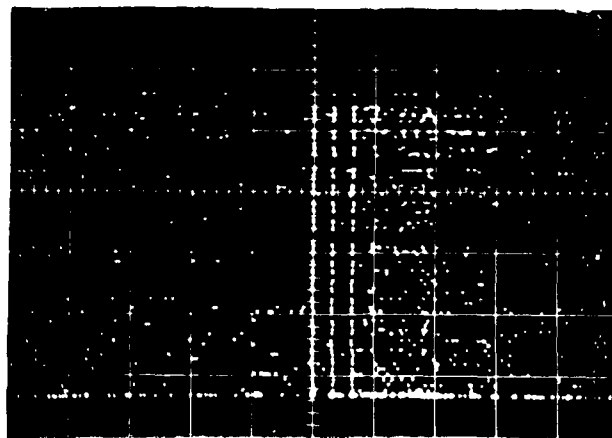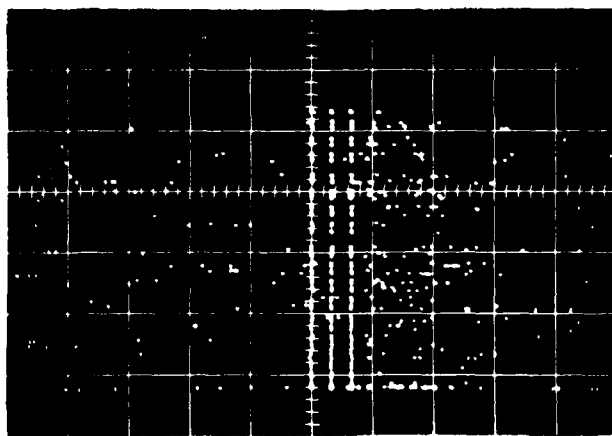
BASELINE
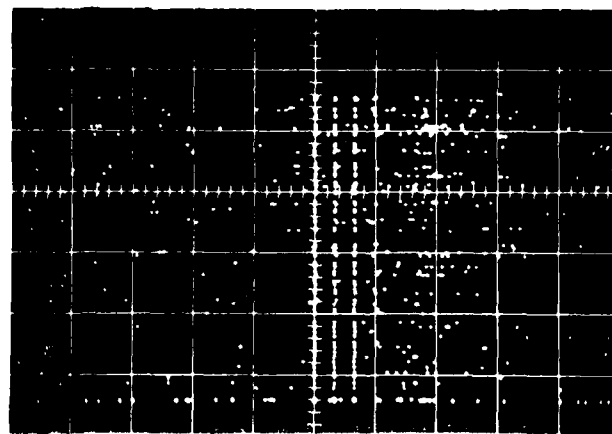
ACUPUNCTURE

RECOVERY

Figure 2 Raster Photographs of Baseline, Acupuncture, and Recovery Periods for a PO Unit. Pulp stimulation was applied at the center of each horizontal sweep. In this case, the stimulus artifacts of each of the three stimuli are visible as parallel columns of dots.

Figure 3. *Time Histogram Profile of PO Unit*

Baseline

Acupuncture

Recovery

Time (msec)

Time (msec)

Time (msec)

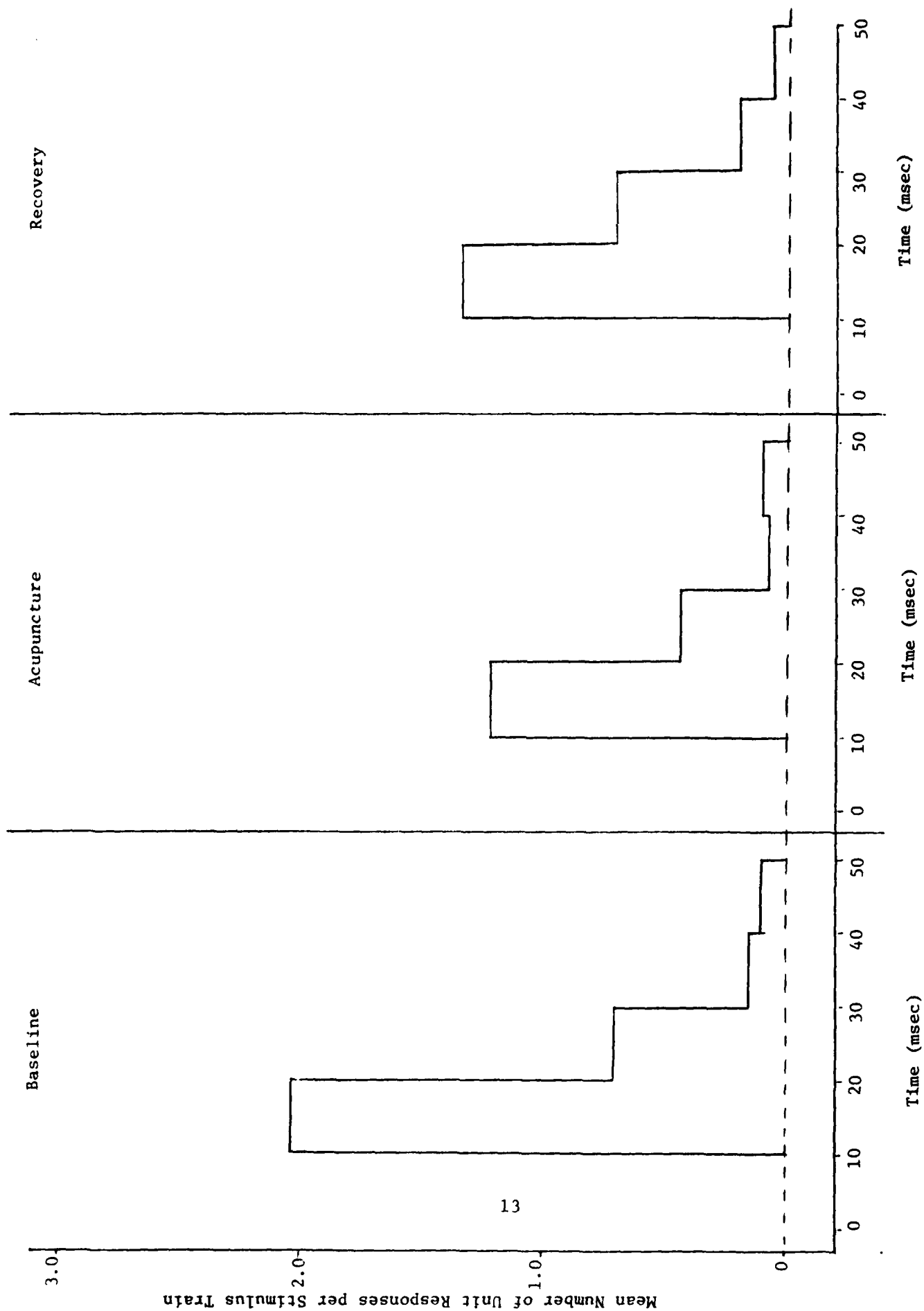Mean Number of Unit Responses per Stimulus Train

3.0

2.0

1.0

0

13

period, and documents the initial stimulus-driven burst-like activity and the subsequent progressive tapering of single-unit response frequency to baseline. The middle third of the figure represents results during Acupuncture stimulation, and shows a significant decrease in both frequency and duration of burst activity. Finally, the right third of the figure represents the summarized results during the recovery phase, which in this particular experiment, reached an intermediate level of recovery over the recording interval examined. It is important to note that initial trials immediately following Acupuncture termination exhibited a significant attenuation of responsiveness, so the lack of full recovery averaged over the entire interval investigated (and including the initial trials) is not particularly surprising. These results are generally typical, although, in some cases, more complete recovery was revealed by the averaged data.

Figure 4 is a Time-Histogram plot summarizing all data from eight experiments in which the entire data collection protocol was completed for PO units. The format of the figure is identical to Figure 3, with the control period to the left, the Acupuncture episode in the center, and the recovery period to the right. The summarized data of all experiments is similar to the example from a single experiment given above. The data from the control period indicates a stimulus-induced high-frequency burst following stimulation, which then tapers to baseline responsiveness over a period of approximately 40 ms. Acupuncture stimulation again imposed a significant attenuation of burst-type activity, although not to the extent seen in the single experiment given above. Also, the single-unit activity is even more depressed during the recovery phase than during Acupuncture stimulation. This is not the result of overall deterioration of the experimental preparation, as documented in a later section. It is a manifestation of a continuation of depression in single-unit activity exhibited during the late stages of Acupuncture stimulation, and the fact that the effects of such stimulation seem to greatly outlast the Acupuncture stimulation interval. However, the most recent data indicates a more rapid recovery than the averaged results due possibly to improvements in our experimental preparation with accumulated experience.

Definitive Acupuncture Data from CM. Although most of our effort in the definitive experiments has been directed to PO units, two experiments have been completed in CM. As with PO, CM is considered to have a powerful association with the aversive aspects of pain (20,33,34). The CM tests involved the same Control-Acupuncture-Recovery experimental format as was used for the PO experiments.

Figure 5 illustrates the nature of the raw dot-raster data from one of the two CM experiments completed, in a fashion similar to the PO data format of Figure 2. The control period (top display) shows the typical burst pattern of thalamic unit responsiveness, exhibiting a high frequency at first which then tapers to baseline levels in 30-40 ms. During the Acupuncture episode (center display), a slight increase in spontaneous activity is seen, but, in spite of this, there is a significant decrease in stimulus-driven burst type activity. Following acupuncture termination, in this example, the data indicates a continuing slight increase in the level of spontaneous activity, while the stimulus related responsiveness of the unit shows full recovery to control conditions. The other CM experiment gave qualitatively
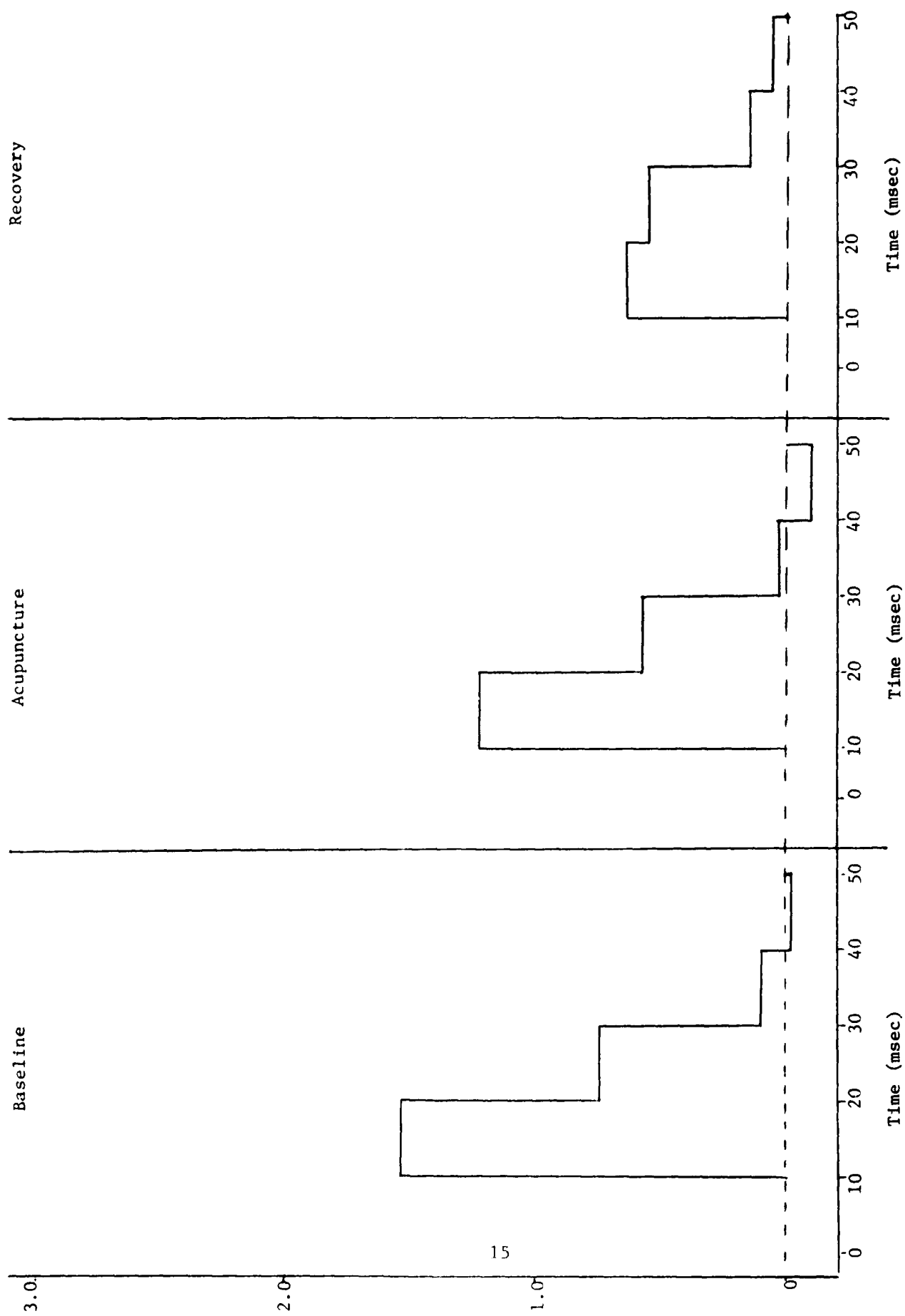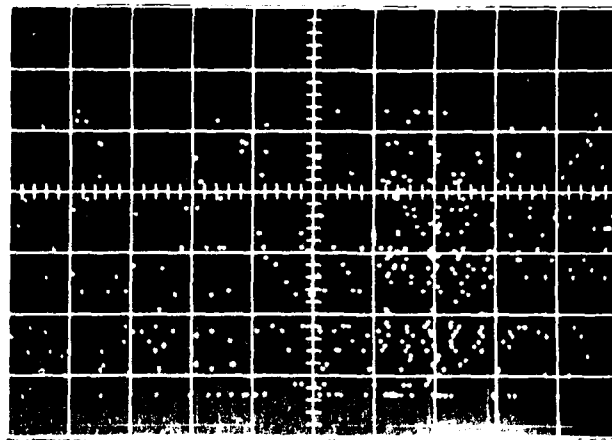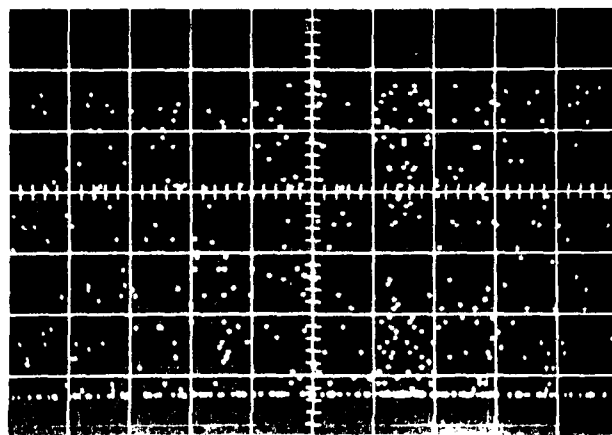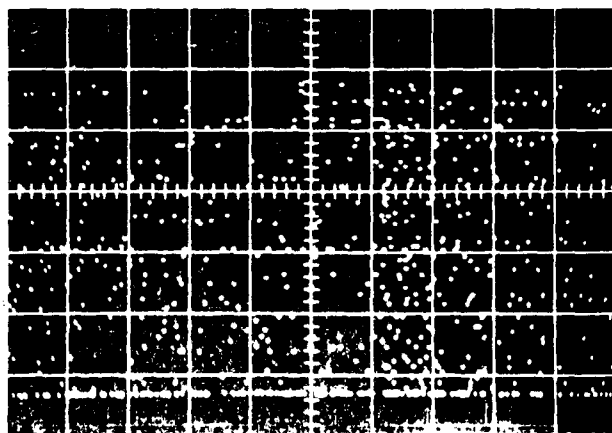
14

Figure 4 Time Histogram Profile for all PO Units (N=8)

BASELINE

ACUPUNCTURE

RECOVERY

Figure 5 Raster Photographs of Baseline, Acupuncture and Recovery Periods for
a CM-Pf Unit. Pulse stimulation was applied beginning at the center
of each horizontal screen. In this case, the stimulus artifacts were
not within the limits of the window discriminator, and were therefore
not recorded.

similar results, with a decline of stimulus-driven activity during the Acupuncture period and a marked tendency for progressive recovery following Acupuncture termination.

A summary of the entire results for the CM experiment of Figure 5 is presented in Figure 6 in Time-Histogram format similar to Figure 3. The baseline period shows the initial stimulus-driven high-frequency burst activity, which then declines in a typical fashion to baseline levels in approximately 50 ms. During Acupuncture stimulation, the data shows a significant attenuation in both burst frequency and duration. Finally, during the recovery phase, the single-unit responsiveness reverts nearly but not completely to the baseline level. As with the PO data, recovery was progressive, and later stages exhibited control-level responsiveness. The PO data indicate that attenuation in responsiveness during Acupuncture or recovery could not be attributed to preparation deterioration, as documented below.

Baseline Stability. It is imperative to answer the question of preparation or baseline stability, because all quantitative interpretation is based on this assumption. Baseline stability refers to the reproducibility of the threshold of the pulp-driven thalamic unit to pulp stimulation over extended periods of time when unencumbered by Acupuncture stimulation.

To examine this problem, we have initiated control sessions of extended lengths to check baseline stability. In general, we have extended many control sessions to as long as 1.5 hours with repeated testing of the threshold of thalamic units. Figure 7 is a diagramatic summarization of the results for the first 25 minutes of the control data (typical of longer durations also) of seven experiments. The data indicates that the threshold of pulp-driven thalamic units is quite stable relative to the magnitude of changes induced during Acupuncture stimulation, so that the model has sound validity. Also, these results were obtained at widely varying intervals following the initiation of the experiment, because of large variations in the time required to set up the electronic instrumentation and to locate and characterize a pulp-driven single unit. Also, in preliminary experiments, it was qualitatively observed that individual units could be held for many hours with only minor alterations in response magnitude. Furthermore, we routinely monitor a battery of physiological parameters during the experiment, and we have seen no significant changes in the physiological state of the preparation. Therefore, changes seen during Acupuncture stimulation episodes or recovery periods cannot be attributed to general preparation deterioration, to unit deterioration, or to progressive loss of the particular thalamic unit due to electrode movement, and such changes are therefore truly a result of the Acupuncture stimulation.

Discussion

The present results demonstrate that single pulp-driven units can be identified in three distinct nuclear groups of the thalamus, as determined by standardized extracellular recording techniques. These locations were identified on the basis of physiological charateristics and stereotaxic locations, as we have not previously had sufficient funding available to include histological verification. The PO units studied exhibited a mean
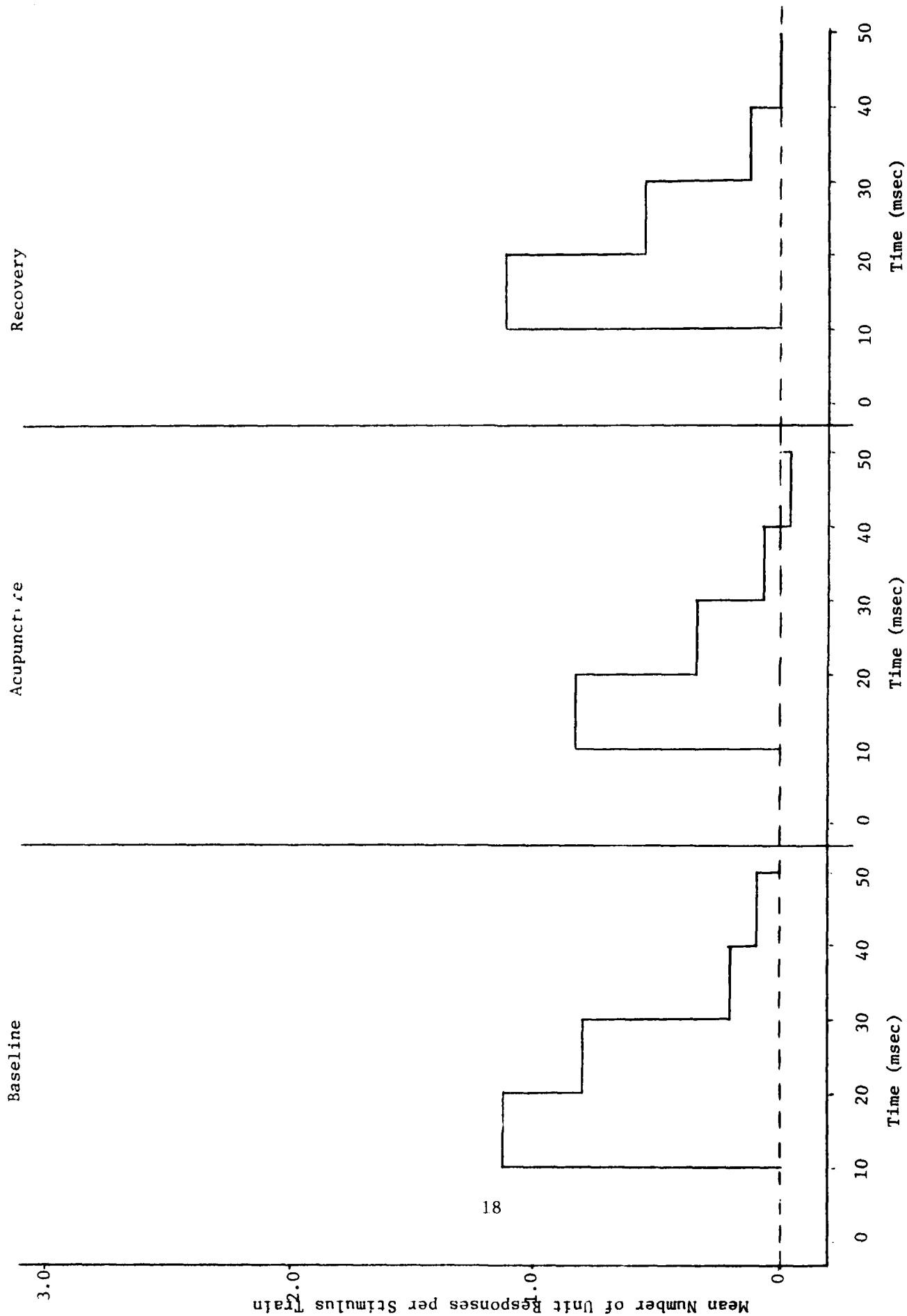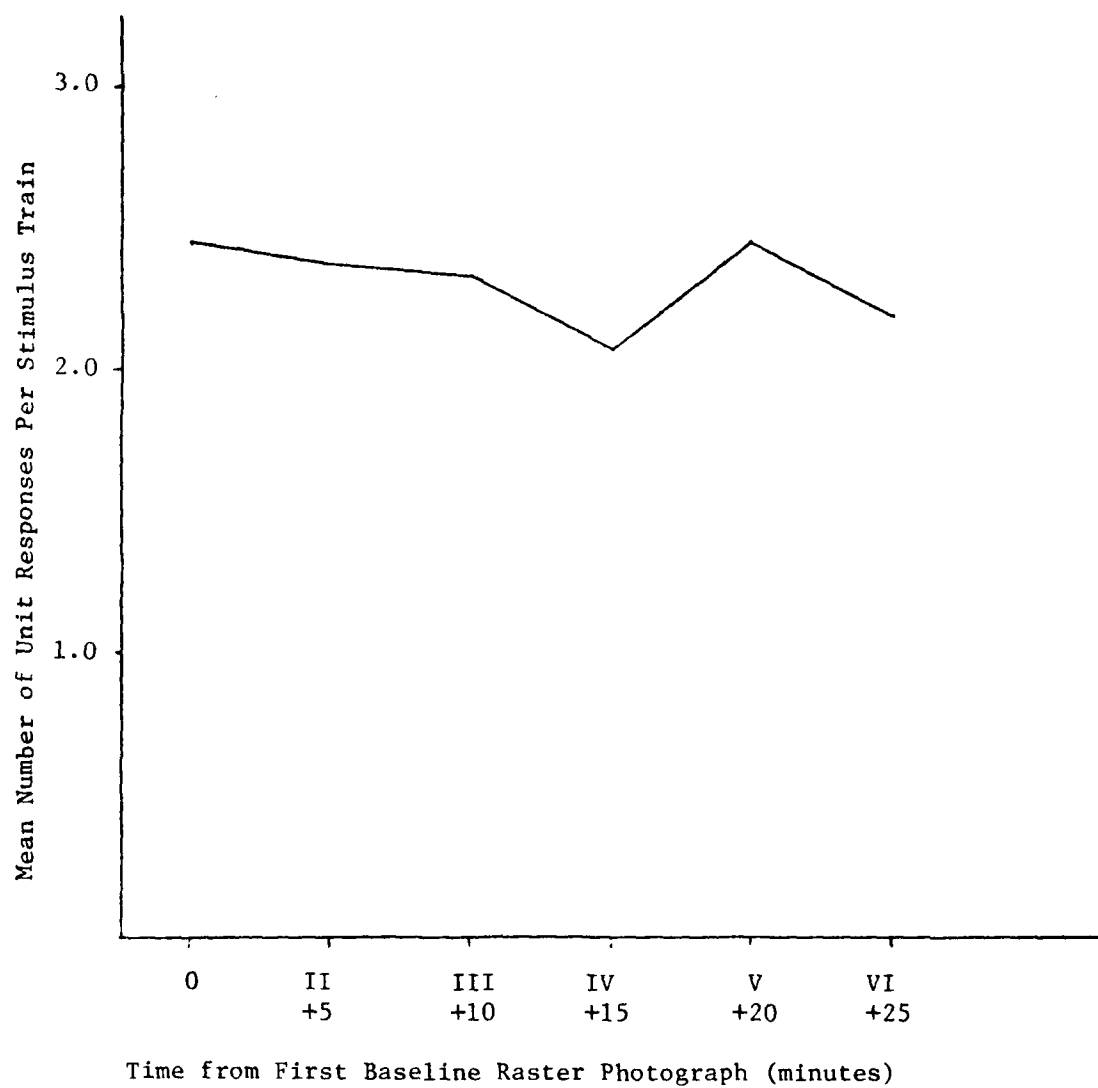
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Figure 6 Time Histogram Profile of CM-Pf Unit.

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Figure 7 Average Baseline PO Unit Activity Over First 30 Minutes of Recording (N=7).

latency of 18.4 ms, a threshold of 140 µa, and an average burst duration
of 2.42 spikes. CM units showed values which were 10-20 percent higher
than PO units in each category, while VB units exhibited the longest laten-
cies and burst durations, but intermediate thresholds. Upon imposition of
Acupuncture stimulation, the majority of the eight PO units and the two CM
units showed a significant and progressive attenuation of stimulus-driven
responsiveness with time, an effect which greatly outlasted the duration of
Acupuncture stimulation. Recovery was progressive, and, in many cases,
after 40-50 minutes, it returned substantially to control levels. These
changes cannot be attributed to loss of the unit or deterioration of the
preparation in general, as verified by the baseline data. Usually, the
recovery periods were not of sufficient duration to permit the demonstra-
tion of complete recovery of the unit, but during the entire recovery
period, the majority of units exhibited a marked progressive tendency to
return to control values. It seems reasonable to assume from the data,
that had longer recovery intervals been included, many of the latter units
would have finally returned to baseline conditions. The spontaneous
activity of the PO and the CM units was not significantly affected, in the
majority of cases, by the application of Acupuncture stimulation.

The thalamic units that have been observed have exhibited small amplitudes
and relatively long latencies. These results probably indicate that the
cells producing these responses are small. Pulpal primary afferents are
known to be principally of the A-delta class (13,16,24,25), and this trend
toward small cells seems to be carried on to the level of the thalamus.
Thresholds for pulp-driven activity were quite high in the thalamus, and the
use of train stimuli was nearly always obligatory. This contrasts with the
lower threshold using short single shocks, which we have shown to be sufficient
for the excitation of pulp-driven units in the Gasserian ganglion (27) and
Trigeminal Complex (44), and emphasizes the necessity of either temporal
and/or spatial convergence for excitation at the level of the thalamus. Data
from the work of others supports the same conclusion (23,31).

The spatial and multimodal convergent properties of the pulp-driven thalamic
units examined to date were not unexpected, as these properties have long
been associated with these thalamic centers (18,19,20,34,45). However,
units of VB have classically been considered the antithesis of these prop-
erties (18), having a precise somatotopic organization in which convergence
from the upper and lower jaw would certainly not be expected. It appears,
then, the pulp-driven units we have found in VB belong to the recently discovered
subset of ventrobasal cells with "extra-lemniscal" properties (20). In common
with extra-lemniscal cells, they show spatial and temporal convergence, low
following rates to repetitive stimulation, and small size.

The feasibility of our conceptual approach is adequately demonstrated by the
present data. The most comprehensive model of pain proposed to date describes
three ascending pathways which directly dictate or modulate the painful exper-
ience (33,34). Furthermore, the proposed systems all have major relays pos-
sessing significant integrative responsibilities at the level of the thalamus.
The anatomical proximity and integrative functions of these systems ascribed
to the thalamus make this level an extremely logical point to define an index
of nociceptive activity indicative of analgesic effects. Therefore, there is
every reason to suppose that our initial hypothesis regarding experimental
model selection was accurate in terms of present experimental knowledge and
theoretical constructs.

CHRONIC NEUROPHYSIOLOGICAL EXPERIMENTS

Chronic (behavioral) experiments are extremely powerful, because they permit the evaluation of central nervous system functions free from complications introduced by anesthetic agents or surgical intervention in Acute experiments and they permit more flexibility than studies in humans. Furthermore, as a component of the overall research program, the Chronic experiments provide the main framework as a link between the Acute and Human experiments. Chronic experiments are of further value because they permit evaluation of Acupuncture effects free of placebo reactions, suggestion, or hypnosis and the flexibility of the imposition of experimental conditions which are not permissible for human studies. They are also required to verify information derived from both Acute and Chronic experiments at the actual level of perception and to obtain data crucial for documentation of safety. The central element of the Chronic Neurophysiological experiments is the development of a model which uses perceptual rather than reflex manifestations of sensation as an index of Acupuncture effectiveness. To adequately study the feasibility of Acupuncture, therefore, a model is required in which the animal repeatedly reports his threshold to pain, a situation which permits optimal monitoring of changes in perception during various control and experimental procedures.

The central features of the Chronic Neurophysiological model are the chronic implantation of electrodes in a tooth pulp to permit application of noxious stimuli, the chronic implantation of electrodes at specific Acupuncture points to evaluate the effectiveness of these procedures, and the use of a perceptual index of the response of the animal to the noxious stimulation.

Tooth pulp is used as the site for the initiation for noxious stimulation for the same reasons as detailed under Acute Neurophysiological experiments and also to provide maximum overlap with the latter experimental model. Tooth stimuli are composed of rectangular pulses of a duration of 0.1 ms. In training sessions involving escape from pulp stimulation, the stimuli are adjusted to an intensity slightly suprathreshold as judged by the animal's behavior. Train stimuli are employed because of the importance, mentioned previously, of allowances for spatial and temporal summation in central pain mechanisms (33-35). The stimulus trains have a duration of 50 ms and a frequency of 50 Hz. This high frequency is required to accentuate perceptual as opposed to reflex responses (e.g., the Jaw Opening Reflex; ref. 46).

Our preliminary Acupuncture stimulation protocols, to be initiated in the immediate future, will initially involve use of the Hoku Acupuncture point. Eventually, all three major Acupuncture points selected for the study (39) will be employed. Electrodes will be placed at the actual Acupuncture point or on the peripheral nerve just proximal to that point. Initial studies will involve use of the best judged waveform based upon the information in the literature (0.1 ms pulses at a frequency of 50 Hz, and an intensity somewhat above perceptual threshold but below levels which induce obvious manifestations of discomfort; refs. 9,12,36). Later studies will be designed to identify optimal waveforms. All experiments will be conducted using standardized psychophysiological stimulation and recording techniques, and will employ the cat as the experimental animal for economy and for maximal correlation to the general pain control literature (18,20,21,34) and previous work in

our laboratory (26-30). To date, the experimental series has involved development of this complicated experimental model, described in subsequent sections of the document. At present, all stages of training and transfer to the Threshold Titration paradigm have been demonstrated to be virtually operational, permitting the initiation of definitive protocols in the immediate future.

Methods

Animal Preparation and Surgery. Experimental animals were initially screened for suitability using a footshock stimulation paradigm (see below), and were then scheduled for surgery. Endotracheal intubation was accomplished using a short-acting barbiturate. Ethrane-Oxygen was administered via a closed inhalation circuit. Upon reaching a level of deep surgical anesthesia, the animal was maintained at that level using Ethrane-N$_2$0-Oxygen throughout the entire surgical procedure. Subsequently, an optically-coupled ECG (to preserve ground isolation) was attached (Terrasyn, Model N-IIIB ECG Isolation Amplifier) and a non-invasive blood pressure monitoring system was applied to one of the anterior limbs (Hoffman-La Roche Arteriosonde, Model 1010). In addition, a Beckman LB-2 Medical Gas Analyzer, sampling the respiratory gases, was employed to continuously monitor end-tidal CO$_2$ as an index of short-term acid-base balance. Respiration was assisted on demand, or controlled, using a Bird Mark IV-VIII Anesthesia Assistor. Intraesophogeal temperature was monitored (YSI Model 43TA) and maintained at 38 $\pm$ 0.5 C by means of a heating pad with thermostatically controlled circulating water (Gaymar Temp-Pump System). The animals were then brought through the surgical recovery process, and allowed several days to recuperate from the surgical procedure. The animals were periodically given physicals, to document the maintenance of long-term health status.

Tooth stimulation electrodes were placed in the experimental tooth (maxillary canine) for the induction of noxious stimulation (17). One electrode was placed in each of two cavities drilled through the enamel to a near pulp exposure. The base of each cavity was filled with silver amalgum, the electrode wire (stranded) was packed in place, and the whole complex was sealed in position using non-conductive adhesive. One of the tooth stimulation electrodes was located on the lingual aspect and one on the buccal aspect of the tooth, both being near to but absolutely distinct from the gingival margin. The wire leads were implanted under a muco-gingival flap, and then directed upward under the facial skin along the surface of the bone between the medial canthus of the eye and the nose, to gain access to an electrical connector mounted in the head pedestal.

The Acupuncture electrodes (implanted during development of surgical techniques) were implanted using adaptations of techniques developed in another laboratory (47). In this procedure, a sleeve (formed by a longitudinal cut in polyethylene tubing) containing an open electrode loop, previously prepared, was placed around the nerve, the sleeve was closed, and, finally, it was sewn to nearby muscular tissue. The Acupuncture lead was directed subcutaneously from the site of nerve placement to the anterior surface of the head, for attachment to the electrical connector of the head pedestal. Extreme care was exercised in

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defining the course of the Acupuncture leads, to minimize lead stress imposed by joint or muscle movements.

As described, both of the tooth and all Acupuncture leads were directed to a head pedestal. The pedestal was built up by repeated applications of dental acrylic, by methods adapted from a report in the literature (48). The acrylic pedestal was held in place by molding it around stainless steel screws mounted in the skull of the animal. The lead wires were connected to a multipin connector complex, which was then embedded in the acrylic pedestal. A flexible cable connected the pedestal plug to a counterbalanced multi-contact commutator, mounted on a floating pulley system so that the commutator floated above the experimental cage to provide minimal restrictions to animal movement.

Stimulation Techniques. The experimental model as described involves two problems related to stimulation, the problem of tooth stimulation and the problem of Acupuncture stimulation. Special considerations related to each of these stimulation techniques are discussed below.

For stimulation of the tooth, an important consideration was the requirement for temporal summation of activity in pain pathways, a phenomena recently realized to be important for nociceptive interpretation in the central nervous system (33-35). We therefore used a train stimulus format, to induce aversive activity in pulpal afferents which was somewhat dispersed in time. Based upon our previous experimental determination of the Strength-Duration curve for pulpal afferents (27), we have chosen 0.1 ms for the width of each rectangular stimulus pulse. The frequency of stimulation which was chosen is that which efficiently activates pulpal afferents, based upon our data from single pulp-driven units in the Gasserian ganglion (30). The latter data indicated that the majority of the units respond faithfully to frequencies up to 300 Hz. We therefore chose a waveform for pulpal stimulation of a 50 ms train of rectangular pulses of 0.1 ms duration at a frequency of 50 Hz, a frequency low enough to insure the activation of the majority of pulp afferents, and yet high enough to permit accentuation of perceptual versus reflex responses.

The problems encountered in Acupuncture stimulation were entirely distinct from those related to tooth pulp stimulation. A variety of waveforms have been described in the literature for both human and animal studies (7,12,36). Waveform is obviously a very important variable for investigation, and many waveforms must eventually be characterized. However, for initial tests of feasibility, we have chosen to use a train of 0.1 ms rectangular pulses at a frequency of 50 Hz, because approximations of this waveform have been shown to work in both chronic animal (9,11) and human (7,12,36) studies, and seems, from our animal data, to be a realistic waveform to activate a majority of afferents. The proper intensity of Acupuncture stimulation is again difficult to judge and is also obviously an important variable for future study. For our preliminary tests in the Chronic Neurophysiological experiments, we have chosen to use an intensity somewhat below that which induced induction of muscle fasiculations visible to the naked eye and, which, in the behaving animal, produced signs of sensation which did not appear to be uncomfortable.

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Training Program. The central feature of the Chronic Neurophysiological
experimental design was the use of a perceptual index to monitor pulp
threshold, the Threshold Titration paradigm of physiological psychology
(49). The complicated nature of the latter procedure necessitated designing
a program in which the animals were conducted through several preliminary
training stages. Following animal selection and surgery, the animals were
initially trained to escape pulp stimulation by pressing a bar. The animals
first learned that once the tooth shock had been initiated, it could be termin-
ated by pressing the experimental response lever (escape behavior). A high
level of escape behavior indicated a great deal of proficiency in the learning
experience, and was used as the criterion for transfer to the final training
stage (Threshold Titration paradigm) to be used for the definitive Acupuncture
experiments. Animals tested to date have exhibited escape behavior in approx-
imately 90 percent of trials after 2-3 training sessions of 80 minutes each.
If the animals exhibited problems in developing an association of the response
lever with pulp stimulation, footshock was alternatively applied as the noxious
stimulus, a situation which provided a high degree of motivation for movement,
thereby affording the animal a greater probability of contacting and learning
the significance of the response lever. Once trained to footshock, such animals
were then generalized to respond to tooth pulp stimulation and reintroduced into
the regular training program. Such generalization has proved to be quite rapid
in a multitude of experimental situations including the present one.

All of the procedures were accomplished in a specially designed experimental
cage, allowing for footshock and access to the pulp and Acupuncture stimulating
circuits, while still permitting maximum animal mobility. The experimental cage
was also designed to eliminate the animals use of the walls to avoid footshock,
to counteract the contingency of the animal continuously holding down the bar
rendering the procedure ineffective, and other experimental problems. The
entire experimental protocol, involving the presentation of stimuli and recording
of responses (escape, avoidance, or failure), were controlled and recorded
entirely automatically. The latter procedure is termed "Automatic Shaping"
(50), described in detail below, an experimental procedure which permits sub-
stantial reductions in personnel committments.

The Threshold Titration paradigm was the final procedure in the experimental
series as designed (49). In the preliminary test of the latter paradigm,
rectangular pulses of 0.1 ms duration were delivered at 1 Hz to the test tooth.
A subthreshold intensity was used initially and repeated a given number of
times (selectable from 1-10), but following every N'th succeeding stimulus, the
intensity was automatically incremented to a new plateau. In the absence of
corrective behavior by the animal, this process continued with equal intensity
increments after every N'th stimulus until noxious or potentially noxious
intensities resulted in immediate bar-pressing responses, thereby resulting
in a lowering of the stimulus intensity. Our very brief experience to date
has demonstrated that the animals will tend to maintain an intensity which is
just below noxious levels for prolonged periods, results which are consistent
with reports in the literature (49), providing a direct measure of perceptual
thresholds to noxious stimulation over extended periods of time. Once the
animals have become accomplished in the Threshold Titration paradigm to tooth
pulp stimulation alone, specific definitive procedures involving concurrent
Acupuncture administration will be introduced. Furthermore, in future experi-

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ments, such procedures can be repeated daily, permitting the accumulation of a voluminous amount of data from each animal in relatively short time periods, thereby greatly reducing the number of animals and time commitment required to generate statistically significant conclusions.

At present, the experimental cage and Automatic Shaping system is completely operational. Also, we have demonstrated the operational status of all training procedures, including transfer from footshock to pulp stimulation, and from pulp stimulation to the Threshold Titration paradigm. We have recently completed the selection and surgical procedures on two new animals, have introduced them into the training program, and plan to initiate definitive Acupuncture protocols in the immediate future.

Experimental Protocol. Basically, three stimulation techniques were employed, footshock, pulp stimulation, and the Threshold Titration paradigm. All procedures were conducted in the same experimental chamber, described in detail under 'Results'.

One session of 60-90 minutes, involving footshock as the aversive stimulus, was used in the selection procedure to evaluate the suitability of a particular animal for introduction into the experimental program. The initial selection procedures were conducted as follows: the animal was placed in the experimental chamber, and the Automatic Shaping equipment (described under 'Results') was engaged. This resulted in the presentation of a train of stimuli to footbars in the lower section of the experimental chamber. The stimuli were continued for two minutes unless the animal pressed the response lever, at which time the footshock was immediately terminated. The footshock was derived from a constant-current stimulus-generator (lab constructed) which supplied a given amount of current regardless of the number of footbars that the animal happened to be straddling. In general, the cats moved aimlessly about at first, but very quickly developed an association between the response and the footshock. Following more trials, they became fairly proficient, even in this initial experimental session, in escaping footshock stimulation once it had been initiated. A signal light warned the animal of an impending stimulus five seconds before it's initiation, and, therefore, the animal had the additional option of avoidance behavior (pressing the bar before the stimulus began). In many cases, the animals effectively learned to avoid stimulation even in this initial selection session. If the animals performance was satisfactory, they were then identified as candidates for surgery, and the surgical procedure was scheduled. The results of our footshock system (successful acquisition of escape and avoidance behavior in 2-3 sessions) compared very favorably to results reported in the literature, which indicate that 5-10 sessions are required for successful training in escape and avoidance (51). Therefore, the present system appears to be extremely efficient.

Following surgery, the animals were introduced to the pulp stimulation paradigm. Pulp stimuli were presented under the same Automatic Shaping format as that used for the initial footshock selection procedures. The pulp stimulation waveform was a 60 ms train of suprathreshold 0.1 ms rectangular pulses at a frequency of 50 Hz, as described previously. The animals were trained in repeated sessions during which continuous monitoring of escape or failure responses was recorded. In this situation the animals were not given the option of avoidance behavior (they weren't warned of an impending stimulus by a signal light), because the

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eventual Threshold Titration paradigm does not permit avoidance behavior and the main purpose of the pulp stimulation procedures are to prepare the animal for the Threshold Titration paradigm. The criterion for success in the pulp stimulation paradigm is performance at virtually 100 percent escape behavior, a situation usually realized after 2-3 experimental ssions.

At the present time, we have demonstrated that all training procedures are operational, that the Threshold Titration procedure is operational, and we are finalizing the facility to initiate definitive experiments using Acupuncture stimulation.

Data Analysis and Interpretation. At present, quantitative data is limited to evaluation of footshock and pulp stimulation training paradigms. Certain footshock data has also been used to evaluate the efficiency of our training procedures (see Table II). Tests of the Threshold Titration data to date are purely of a qualitative nature, although definitive protocols are to be initiated in the immediate future. The definitive data will be analyzed to quantitatively assess the perceptual efficacy of the various Acupuncture administration strategies in terms of alteration of sensory threshold. Particular attention will also be given to induction and recovery profiles and to indications of physiological and psychological safety, as judged by general observations and specific definitive data and, possibly the eventual monitoring of vital signs using transducer implants. Data related to physiological and psychological safety, in conjunction with post-lesion data regarding pathways and mechanisms, will be instrumental in the definition of permissible limits of safety for human studies and to obtain FDA sanction of successful therapeutic procedures.

Results

Development of the Experimental Facility. During the present reporting period, we have engaged in a continuous process to upgrade various facets of the experimental program, until at present we have arrived at a facility which is essentially operational. Three main problems received particular attention, testing of the Threshold Titration Generator, further development of the Automatic Shaping experimental procedures and the complete redesign and construction of an entirely new experimental chamber. Discussions related to the Threshold Titration Generator and the experimental chamber are discussed in following paragraphs. The work related to Automatic Shaping procedures involves the presentation of detailed experimental data, and the discussion of this topic is deferred to a later section.

During the initial portion of 1975, our consultant electronic engineer designed and constructed a Threshold Titration Generator to our specifications. The specifications included the ability for the stimulator to follow a trigger input, the inclusion of photic isolation of output circuitry from the trigger input, constant-current output charateristics, an output range of 0-100 μa accurate to within 1 percent, the inclusion of an additional output independent of the stimulus output for purposes of recording stimulus intensity, the inclusion of an intensity-stepping logic operating from the trigger input having 32 equal intensity steps, provisions to control the maximum intensity of the stepping series over the range 0-100 μa, and provisions for an input from the response lever in the experimental chamber which also exerts appropriate

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influence on the stepping logic. At the beginning of the present reporting period, the Threshold Titration Generator was tested, and after adjustments and modifications, its ability to accomplish its assigned task was verified. Also, at that time, we ordered a chart recorder (Omniscribe, Houston Instruments), and upon its receipt, we have tested the recorder itself and the Threshold Titration Stimulator-chart recorder combination. The testing procedure consisted of triggering the Threshold Titration Generator (Grass S-88 Physiological Stimulator), and verifying that the Generator output was correct and accurate and that the chart recorder accurately recorded the occurrence and intensity of individual stimuli.

Experience with the original experimental chamber revealed several major deficiencies. It did not allow for sufficient vertical movement of the animals, and resulted in contact between the top cover of the chamber and the implanted head pedestal. This, combined with rotational movements of the animal, eventually resulted in lead entanglement and dislodgement of the head pedestal. Also, all walls of the experimental chamber were made of plexiglass, and at times provided a safe, non-conductive surface for the cat permitting his identification of "safe spots". Furthermore, the previous chamber was inappropriately designed to allow for the handling of animal waste products.

After a thorough review of all data and subjective observations, a new experimental chamber was designed and constructed. The new chamber is composed of 18 guage stainless steel and 3/8" plexiglass (Figure 8). The total height is approximately 39", with a distance of 34" between the top of the shock grids and the ceiling of the chamber. The interior is approximately 11" wide and 27" long. The top and right side (as viewed from the front of the figure) are made of 3/8" plexiglass, in order to permit observation of the experimental animal. An 8" long, 3/4" diameter horizontal aluminum bar (the response bar or manipulandum) is mounted on the right wall, 7" from the base of the chamber. Access to the chamber is possible through a 19" X 25" door, hinged on the left side of the front wall. The foot stimulation grids (1/2" aluminum rods with a center to center spacing of 1") are set at each end into a 1/2" thick teflon block (nonconductive), and the entire grid assembly can be removed for cleaning. The left wall, which is opposite the response lever, can be repositioned in order to reduce the interior dimensions of the chamber. For the present study, it was placed at approximately 14" from the right wall of the chamber. The bottom of the enclosure is made to accomodate a tray for animal wastes. The entire chamber is assembled with nylon nuts, bolts and washers. Also, nylon strips, 1/16" thick, are inserted between the contacting surfaces of the individual walls. Thus, each wall can be energized, permitting the elimination of safe areas within the chamber during electric shock by appropriate contingency arrangements in the Automatic Shaping program. An electric fan (Amphenol, 760-126LF182), rated at 30 CFM (cubic feet per minute) is mounted in the center of the left wall, approximately 4" from the top of the chamber. A long and very flexible cable communicates between the head pedestal and a counterbalanced multicontact commutator, mounted on a pulley system above the ceiling of the cage proper. This arrangement effectively prevents entanglement of lead wires, even in light of gross vertical and rotational movements of the experimental animal.
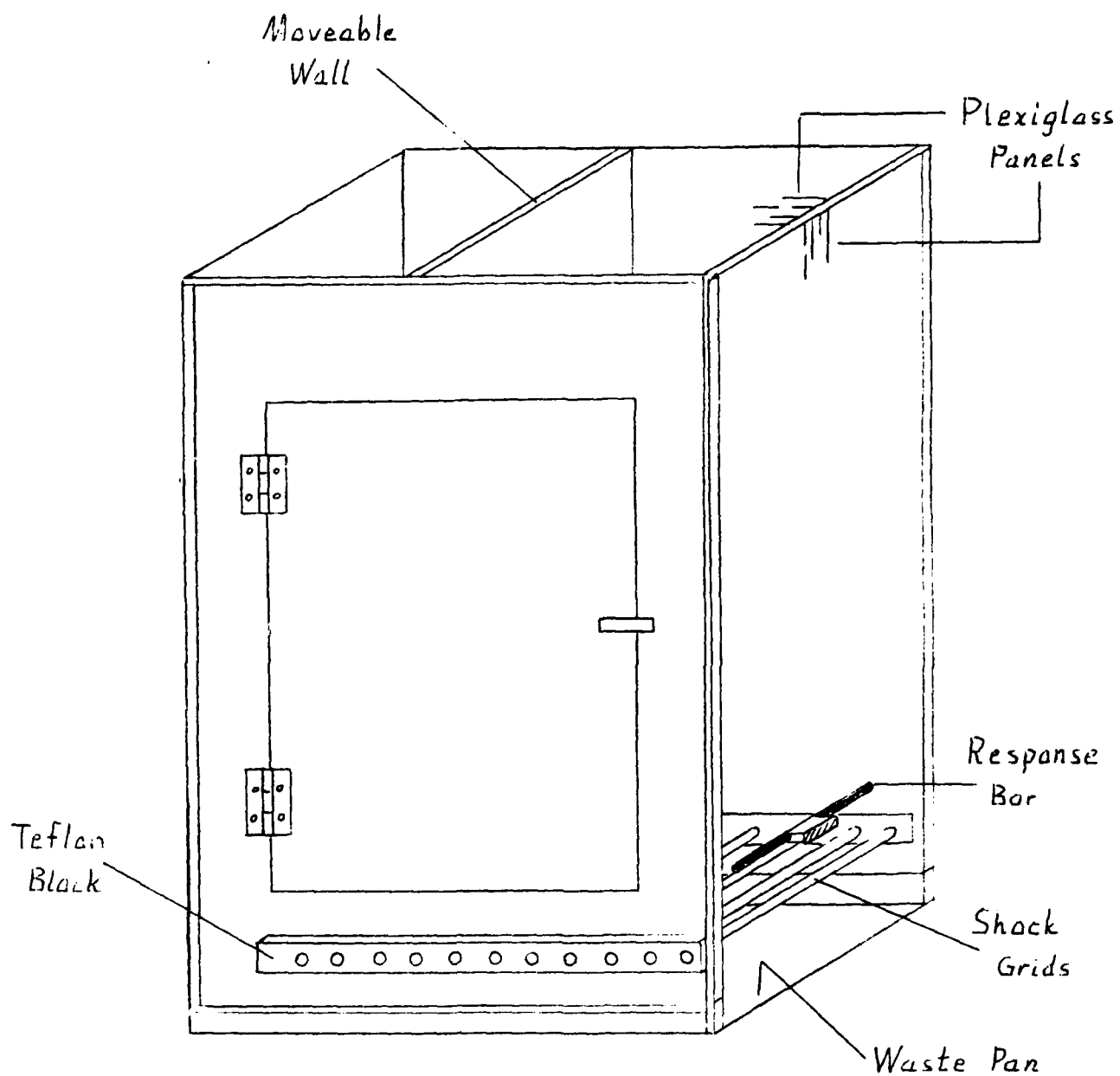
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Figure 8. Experimental Chamber for the Chronic Neurophysiological Experiments.

At present, each major component of the facility has been individually brought to operational status. Also, various component combinations have been tested, and we have verified full operational status of the facility through all stages of animal selection and training. In addition, we have tested the Threshold Titration paradigm. The preliminary tests indicate that the latter paradigm is tentatively operational, but some preliminary modifications of the system may be required before we can initiate definitive Acupuncture protocols.

Experimental Results. Our experimental results fall into five categories: Automatic Shaping, a complete reorganization of the various facets of the selection and training procedures, quantitative testing of the implant circuitry of the cat and subjective results of pulp testing, behavioral modification from footshock to pulp stimulation as the aversive stimulus, and behavioral modification from pulp stimulation to the Threshold Titration paradigm.

The entire selection and training procedure is dependent upon the Automatic Shaping equipment complex, which we have assembled and integrated into the program. Two types of behavior are involved, escape behavior (the animal terminates shock once it is initiated) and avoidance behavior (the animal responds such that the stimulation is avoided altogether). In an individual experimental session which contains a series of stimulus presentation episodes, several time intervals are important: a), the inter-trial interval (ITI), the interval between the termination of the aversive stimulus and the beginning of the next stimulus trial; b), the unconditioned stimulus (US), the application of a stimulus of interest, in this case, the aversive stimulus; and, c), the conditioned stimulus (CS), in our case, a signal light, which warns the animal of an impending stimulus. The CS is used only when avoidance behavior is permitted.

The control and operation of the conditioning periods are accomplished by the use of front-patched general control equipment for behavioral research (in this case, built in-house at the Ames Research Center, NASA). The component module array has been selected to program an escape-avoidance schedule with limited hold (in the case of no response, the stimulus is turned off after the US interval). The sequencing and allowances for various behavioral contingencies is controlled by a series of clocks and timers which activate appropriate relays. The relays, in turn, permit the application of appropriate voltages for the presentation of the noxious stimulus (US), activation of the signal light (CS), or initiation of an ITI.

In the footshock paradigm, we use all three time intervals (CS, US, and ITI). The CS, permitting avoidance behavior, is included because it provides a better index of the effectiveness of our escape training program. In the later stages of training involving pulp stimulation, the possibility for avoidance behavior (CS) is not included, because it is desired at this point to shape the animal's behavior toward the Threshold Titration paradigm, and the latter paradigm does not involve the use of a CS. Therefore, in the pulp stimulation paradigm, an ITI leads directly to a CS. Four electromechanical counters are connected so that the total number of lever presses, avoidance responses, escape responses, and failures to terminate shock are automatically recorded. Each experimental session lasts 80 minutes, and the CS, US and ITI durations are five seconds,

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1 minute, and 45 seconds, respectively. Shock intensity is set at approximately 0.5 ma (footshock) or at approximately 20 μa (pulp training), and can be varied over the range of 0-100 μa (as the animal permits) in the Threshold Titration paradigm.

To summarize the training sequence, in the case of avoidance training, a trial begins with CS onset (the signal light). If a response occurs within five seconds (avoidance behavior), the CS is turned off and ITI begins (45 seconds). If no response occurs within five seconds, the US is presented (while the CS remains on). Both the US and CS are then terminated by a response (escape behavior), or, alternatively, by time-out of the US interval (one minute). In the case of escape training in the absence of the avoidance response possibility, the CS is bypassed, but otherwise the format is similar to the avoidance paradigm.

Table II shows data from the first trial of Automatic Shaping, the session used for animal preselection. In this case, avoidance behavior is permitted, and, therefore, the CS condition is included. The data represents results from 15 different animals. The data shows that most animals exhibit a significant amount of escape behavior and some exhibit a tendency to begin to respond to the avoidance format, even at this early point in the program. Alternatively, the performance level of a few animals was very poor. Based upon our experience, we would judge 11 of the 15 animals to have been successful in this first (selection) session, with animals number 3, 5, 6 and 9 being rejected based upon poor performance.

In our initial experiments to establish optimal protocols to train and maintain each animal, it was necessary to subject each animal to many experimental sessions. Preliminary information regarding some of the latter results was reported in the last report of June 30, 1975 (52). These training procedures allowed provisions for both escape and avoidance behavior. The following parameters had to be assessed: duration of session, number of session, box dimensions, level dimensions, response lever location, number of trials required to reach successful levels of escape performance, and resistance to extension (strength of the learned association). This information was necessary to properly design future protocols. These various parameters have now been established. The interior dimensions and lever size and location in the redesigned experimental chamber have been described in this document. It has been found that 2-3 sessions of 80 minutes each are required for the animals to successively acquire escape performance, and, once trained, the animals can be maintained with one session per month.

Our original experimental plan was to initially train animals in the footshock paradigm, and, once successfully trained, conduct the experimental surgery, re-establish the footshock training, transfer the animals to pulp-stimulation, and finally transfer the animals to the Threshold Titration paradigm. The program proved unreliable, because of the serious contingency of loss of animals during surgery and inefficiencies in training due to imposition of the surgical time interval. In the initial portions of the present year, we tested various other combinations. Based upon this information, we have now selected the following procedure. The animals are initially subjected to an experimental session involving footshock as the aversive stimulus, permitting

TABLE II

Number	UOHSC Number	Trials	Escapes	Avoidance	No-Response
1	5796	54	39	0	15
2	5717	99	96	1	2
3	5757	41	4	0	37
4	5756	70	63	1	6
5	5764	43	7	0	36
6	5777	51	0	0	51
7	5779	57	55	2	0
8	504	132	92	37	3
9	503	50	7	0	43
10	5723	85	80	4	1
11	5390	149	142	7	0
12	3964	135	133	2	0
13	5802	83	81	1	1
14	5795	58	36	4	18
15	507	94	76	0	18

Data using the Automatic Shaping program from the first (animal selection) experimental session. Based on the data, animals 3, 5, 6, and 9 were rejected based upon unsatisfactory performance.

animal pre-selection and the elimination of initial erratic behavior. The
animals are then scheduled for experimental surgery, and, upon recovery, are
trained directly in the tooth pulp paradigm (or footshock paradigm, if neces-
sary for initial orientation), and the behavior is then modified to the Thres-
hold Titration paradigm. This overall program eliminates the serious contingencies
of animal loss during surgery, allows preselection of suitable animals, and
training is rapid and efficient. Training is made much more efficient by improve-
ments in the facility (see above) and by the fact that there is more temporal
continuity in the various training stages. Therefore, based on extensive tests,
mostly using the footshock paradigm (because this required no prior surgery) but
also using pulp stimulation, we have now adopted this format for the overall
program of animal selection, preparation and training.

Details regarding the integrity (continuity) and temporal stability of the
chronic tooth electrode preparation is extremely important. Therefore, repeated
measures of the impedance of the electrical circuit to the tooth were conducted
in several different animals. The results in three animals indicated an average
impedance of 21, 23, and 27 kΩ, respectively. Each of these preparations
indicated acceptable temporal stability. The test procedures were repeated at
least 8 times, with a minimum interval of 1 week between individual tests in
each animal. The resultant values in each animal were stable to within approx-
imately $\pm$ 10 percent. This correlates well with values indicated in the
literature (17). The circuit to the tooth was judged to be intact, because
single-shock rectangular pulses of 50 μa induced jaw-jerk reflexes and signs
of discomfort. No other responses were noted. Involvement of the periodontal
structures or any other tissues are ruled out, even if a leakage of current
had occurred at the electrode site or at some other point due to a break in
the leads, because the current level employed was too low to stimulate anything
but the very specialized structure of the tooth (53).

After bringing all facets of the total experimental program that had been developed
to apparently operational status, we placed the experimental animal in the
chamber and tested the pulp stimulation circuit physiologically. The animal
had previously been trained to a high level of competance in the footshock
experimental paradigm. Initially, with the animal out of the experimental
chamber and using manual operation of a physiological stimulator, a level of
stimulation was identified which was definitely suprathreshold for pulpal pain.
The stimulus waveform employed was a 50 ms train of 0.1 ms rectangular pulses
at a frequency of 10 Hz. The animal was noted to respond visibly to all stimulus
presentations. However, it was also noticed that there was some fasciculation
of facial musculature and some jaw-jerk responses accompanying behavioral
reactions to the stimulation. At this time, the frequency of stimulation was
increased to 50 Hz, with other stimulus parameters being held constant. Using
the latter frequency, behavioral reactions reminiscent of discomfort were
obtained, but there was not visible evidence of fasciculation of facial or
neck musculature. This confirms evidence from our laboratory (54) and from
the general literature (47) that the relative manifestation of perceptual
versus reflex effects is proportional to the frequency of stimulation, and
the use of a stimulus train of a sufficiently high frequency provides a
situation such that perceptual thresholds are markedly less than the thresholds
for reflex responses. The present qualitative experiment also served to
provide a dramatic demonstration of the effectiveness of the tooth pulp

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preparation using physiological criteria, in substantiation of the abovementioned data based upon electronic characterizations.

Following development of the previously mentioned facets of the experimental program, the test animal was placed in the experimental chamber to initially test the tooth pulp stimulation paradigm. The particular animal tested was one in which training had been accomplished to a high level of competance in the footshock paradigm. Initially, the threshold to pulp stimulation was approximated, and the intensity of stimulation was set to a value significantly (50 percent) higher. The stimulation waveform employed was a 50 ms train of 0.1 ms rectangular pulses at a frequency of 50 Hz for this and subsequent tests, based upon the qualitative pulp-test data previously described. The animal exhibited escape responses on all of these preliminary trials. Subsequently, the intensity of stimulation was lowered considerably (well below threshold for any visible behavioral manifestation), and a stimulus episode was applied once per second, with every fourth stimulus being incremented in intensity by a slight amount. The animal began responding to stimulation by pressing the response lever at approximately 2 volts, and, so, as a reward, the intensity was lowered slightly. However, after every fourth stimulus presentation, the stimulus intensity was slightly increased again, and every time the intensity approached intensities of approximately 2 volts, the animal would again press the response lever. Occassionally, the animal would allow the intensity to climb slightly above 2 volts, but in no case would he allow it to get above 2.5 volts. This procedure was repeated approximately ten times. The results demonstrate an extremely efficient transfer of behavioral reactions from the pulp stimulation paradigm to the Threshold Titration paradigm; once the intensity of stimulation had reached perceptual or noxious thresholds (or perhaps just perceptual thresholds in these initial tests) based upon behavioral observations, the animal responded to every trial before the CS timed-out. Not one failure to exhibit the escape response was noted, once the region of apparent perceptual threshold had been obtained.

The present data strongly supports the feasibility of the pulp stimulation model, substantiating previous data. Furthermore, the present data provides a powerful demonstration of the feasibility of the Threshold Titration paradigm. This has been the major goal sought in the Chronic Neurophysiological experiments for the present point in time. As the next step in this experimental series, preliminary experiments are to be initiated immediately in the Threshold Titration paradigm, to permit technical adjustments of the procedures, and then definitive Threshold Titration protocols will be introduced. The latter definitive experiments will include Acupuncture stimulation protocols: experimental animals with Acupuncture electrode implants at the Hoku point are now being prepared.

Discussion

Several key goals regarding the experimental facility and the animal surgical and training procedures have been accomplished. The Titration Threshold Generator was designed and constructed, and following adjustments and the addition of a suitable recorder, the system was brought to a status of complete readiness for the experimental procedures. Concurrently, Automatic Shaping equipment was acquired, integrated into our system, tested on a practical basis for general

suitability, and adapted to our specific experimental needs. Also, an entirely
new experimental chamber was fabricated, a device which alleviated many of the
serious limitations of the old chamber. We have also redesigned the entire
experimental program, introduced a specific procedure allowing preselection of
appropriate animals, and improved our surgical techniques through further
experience and consultation with other laboratories. At the present time, the
entire program of animal selection, surgical preparation, and training is
extremely rapid and efficient. Finally, we have demonstrated all independent
steps in the total procedure and all behavioral modifications required for the
transfer from one procedure to another, and all stages of the program to prepare
an animal for definitive studies appear efficient and reliable.

The use of the Automatic Shaping procedure is a prominent element in our
experimental program, because of reduced commitments of personnel afforded
by this procedure and definite improvements in the rate of establishment of
conditioned responses. The conditioning and training of cats to avoid an
electrical shock by performing some response such as pressing a lever is a
valuable technique for the study of motivation and behavior (55). At the same
time, such avoidance and escape training has provided a useful model for
measuring the sensitivity of animals to electric shocks administered through
the footpads (or other sources) and for observing the alteration of aversive
thresholds through the administration of anesthetics (51). Typically, a
procedure required for training the animals to perform the specific response
(operant) is referred to as shaping, and involves the *systematic reinforcement*
of behaviors which at first may be dissimilar to the desired operant, but,
which may be required to orient the subject toward the lever or toward a
particular area in the experimental chamber. Successive behaviors in the
program are then conditioned which more and more approximate the desired response.
Finally, the actual operant itself is performed. This procedure is tedious and
time-consuming, and often not compatible with the total experimental design (56)
Successive approximation training or "shaping" to escape footshock or other
aversive stimuli by pressing a lever can require as many as fifteen hours for
each animal (51).

An abbreviated and automatic method for escape and avoidance conditioning,
which circumvents the problems inherent in the basic shaping procedure, is
also available (50). Automatic Shaping eliminates the rigorous successive
approximation format, and significantly reduces the time necessary for condi-
tioning. The experimental environment is structured such that the probability
of eliciting the desired response from the animal is maximized. For example,
if the interior dimensions of the response chamber and the location and size
of the response lever are correctly determined, then on the first conditioning
trial, the animal will display exaggerated motor activity in response to e.g.
footshock, and this activity will lead to contact with the lever and termina-
tion of the shock. Following a brief number of trials, the desired operant
is performed with regularity. Furthermore, the system can be automated.

Several aspects of the described Automatic Shaping data, backed up by our
qualitative observations, emphasize specific advantages of the present Auto-
matic Shaping program. Firstly, the present data shows that one session (the
selection session) of Automatic Shaping to escape or avoid footshock will be
adequate to establish the association between a bar press and shock termination.

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Secondly, the performance level during the first session can reliably be used to select suitable experimental animals prior to the surgical procedure, thus reducing the risk of committing the time, personnel, and expense of preparation of an animal which, at best, would be difficult and costly to train at a later date. Furthermore, subjective observations provide definite evidence that there is a significant decrease in jumping and struggling of the experimental animals during the initial test trials as the first session progresses. This is very desirable, because once the head pedestal and associated lead wires are implanted in the cat, exaggerated activity would increase the probability of damaging the preparation. Also, a more sustained orientation toward the response lever is seen as the first session continues, and thus the animal is preconditioned to a more suitable position in the experimental chamber in preparation for later transfer to tooth pulp stimulation and subsequent stages of training.

The desired experimental operant in our program is the Threshold Titration paradigm, previously described in detail. The Automatic Shaping procedure described above has been adapted from the basic considerations in the literature to the peculiar aspects of our particular experimental situation which have been identified experimentally. Our data indicates very efficient training, using the Automatic Shaping protocols, through all stages of the selection and training sequence to the final operant of the Threshold Titration procedure. Thus, except for minor adjustments of the Threshold Titration procedure, it is considered that the entire facility including the animal model, instrumentation, and techniques is operational at the present time. The initiation of definitive experiments, including Acupuncture stimulation protocols, are expected in the immediate future.

SUMMARY AND CONCLUSIONS

1. Two definitive experimental programs have been initiated to characterize
    the feasibility of Acupuncture Analgesia under conditions amenable to
    portable use in the field. The two experimental programs are designated
    the Acute Neurophysiological Experiments and the Chronic (Behavioral)
    Neurophysiological Experiments, respectively.

2. The Acute Neurophysiological Experiments are based upon recordings from
    single neurons in three thalamic nuclear integrative centers which have
    been implicated as having significant contributions to pain mechanisms,
    and the effects that concurrent Acupuncture stimulation protocols exert
    on behavior of these thalamic units. The results to date include:

    a) continued development of the experimental facility and equipment
        array permitting single unit recordings;

    b) thalamic mapping procedures using single-unit recordings to
        characterize baseline properties of units in three nuclear
        centers of interest, using tooth pulp stimulation as the source
        of noxious activity;

    c) definitive experiments (in progress) to quantitatively characterize
        the effects of Acupuncture stimulation on pulp-driven unit activity
        in the thalamus. The results indicate that Acupuncture stimulation
        significantly attenuates pulp-driven units believed to be involved
        in the aversive components of pain perception.

3. The Behavioral Experiments are based on the Threshold Titration paradigm,
    in which experimental animals having chronic electrodes implanted in the
    tooth pulp (source of noxious activity) and near Acupuncture points are
    trained to regulate the level of pulp stimulation to levels very near
    threshold for extended time periods. This permits long-term monitoring
    of the efficacy of pain perception (not reflex responses!) during various
    control and Acupuncture administration protocols. The results to date
    include:

    a) redesign and construction of an experimental chamber to optimally
        circumvent all contingencies identified in previous experiments;

    b) complete development of training procedures such that an experi-
        mental animal will terminate an aversive stimulus (either foot-
        shock or pulp stimulation) by pressing a lever (animal selection
        and training program);

    c) initial tests to verify that the behavior of an animal successfully
        trained to terminate pulp stimulation can be quickly and efficiently
        transfered to the Threshold Titration experimental paradigm, the
        procedure to be used in definitive experiments to quantitatively
        evaluate the efficacy and mechanics of Acupuncture.

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